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INDO-PACIFIC FISHERY COMMISSION

Papers presented at the

EIGHTH SESSION OF THE INDO-PACIFIC FISHERY COMMISSION WORKING PARTY ON FISH TECHNOLOGY AND MARKETING

Yogjakarta, Indonesia, 24-27 September 1991

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome 1992

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PREPARATION OF THIS DOCUMENT

The supplement to the report of the Eighth Session of the Indo-Pacific Fishery Commission (IPFC) Working Party on Fish Technology and Marketing contains all the research contributions which were presented and discussed at the Session. The papers have been edited by David James, Technical Secretary of the Working Party, but the references have not been checked for conformity with the standard FAO format.

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ABSTRACT

The publication contains the forty-seven papers containing the results of research undertaken in the Indo-Pacific Fishery Commission (IPFC) region as part of the programme of research cooperation of the IPFC Working Party on Fish Technology and Marketing. The papers were presented and discussed at the eighth session of the Working Party held in Yogjakarta, Indonesia, from 24-27 September 1991. They have been divided into four sections: shrimp microbiology and processing; fermented fish products and fish silage; fish handling and processing and salted and dried fish, and product development and testing.

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BACTERIOLOGY OF TROPICAL SHRIMP AND MARINE SEDIMENTS

by

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ABSTRACT

Freshly harvested shrimp had bacterial counts ranging from 10^4-10^6. *Klebsiella and* Enterobacter were the predominant coliforms present in fresh shrimp and accounted for the positive faecal coliform tests. These coliforms were also present in sediments from shrimp harvesting areas. Potentially pathogenic vibrios like *Vibrio parahaemolyticus* and *V. vulnificus* were present in freshly harvested shrimps though *V. cholerae* and Salmonella were absent.

INTRODUCTION

Bacteriology of tropical shrimp is of considerable interest because of the importance of this commodity in international trade. Though a number of studies have addressed the bacteriological spoilage of shrimp and bacterial counts in shrimp after landing, there is paucity of information on the bacteriology of freshly harvested shrimp and their environment. This information would help us to know which groups of bacteria are present in shrimp in their natural environment and in what proportions. Such data is essential to decide which bacteria are contaminants, introduced into shrimp during handling and processing. Cann (1977) reviewed the bacteriology of tropical shrimp and pointed out that most of the data available are on freshly landed shrimp and that it is difficult to exclude the effect of handling on the microflora.

The FAO initiated a project to define the bacteriological status of freshly harvested tropical shrimp and to understand whether pathogens like *V. cholerae* and *Salmonella* would be present in the environment in which the shrimp live. The results presented here are part of such a study on marine shrimp harvested off Mangalore, South West Coast of India.

MATERIALS AND METHODS

Shrimp and sediments:

Samples of freshly caught shrimps were collected on board commercial trawlers. As soon as the net was hauled, shrimps were transferred to sterile plastic bags using sterile forceps. The bags were sealed and cooled without direct contact with ice in an insulated box and transferred to the laboratory within 2 hours. Samples of sediments were collected using a grab and transferred to sterile containers on board the vessels. A total of 40 samples of shrimps and 35 samples of sediments were examined.

Analysis:

Total plate count, faecal coliform plate count and detection of *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *Salmonella* were done using standard procedures (FDA, 1984). Samples testing positive for faecal coliforms were analyzed for the presence of various enterobacteriaceae as follows. Tubes of EC broth showing gas at 44.5°C were subcultured to Eosin Methylene Blue Agar and 5 colonies from each sample were subjected to biochemical tests as described by MacFaddin (1980).

RESULTS AND DISCUSSION

Bacteriological profiles of some of the representative samples of shrimps and sediments are presented in Tables 1 and 3 respectively. Bacteriological counts of freshly caught shrimps ranged from

10^4-10^6. The majority of the samples (22) showed counts around 10^5 with a few around 10^4 (10 samples) and 10^6 (8 samples). Most of the earlier reports (Venkatraman *et al*, 1953, Jacob *et al*, 1962, Cann, 1977 and Nirmalathampuran *et al*, 1983) indicate that bacterial counts in freshly landed tropical shrimp range from 10^3-10^7/g. This study indicates that as the shrimp leave the water, the count would be between 10^4-10^6 with 10^5 being the most frequently encountered value. The counts observed in this study are about one log unit higher than that reported for shrimp from temperate waters. Hobbs *et al* (1971) and Cann *et al* (1971) noted total bacterial counts in freshly caught scampi (*Nephrops norvegicus*) to be 10^4 and 10^3/g at 10°C and 37°C respectively.

Interestingly, as shown in Table 1, many samples of freshly harvested shrimp showed the presence of faecal coliforms. Further analysis of these faecal coliforms indicated that most were not *E. coli* (Table 2). Recently there have been reports to indicate that in tropical waters only about 30% of faecal coliforms turn out to be *E. coli*, whereas in temperate waters the confirmation rate is over 90% (Bermudez and Hazen, 1988; Jeyasekaran *et al* (1991). As shown in Table 2, the positivity in the faecal coliform test was mostly due to *Klebsiella* and *Citrobacter* groups, capable of growing at 44°C, are present. *Klebsiella pneumoniae* has been shown to colonize natural environments (Duncan and Razzell, 1972). The results presented here support these earlier observations and also indicate that even in the marine environment, *Klebsiella* and *Enterobacter* might survive and may even colonise the marine sediment. This suggestion is further supported by results presented in Table 4 which show that these organisms are indeed detectable in the marine sediments.

Results in Table 2 also indicate that though *V. cholerae* and *Salmonella* were absent in freshly harvested shrimp, potentially pathogenic vibrios like *V. parahaemolyticus* and *V. vulnificus* were present. Recent research from a number of laboratories in different parts of the world suggest that pathogenic vibrios are native to the brackish/coastal marine environments (Colwell, 1984). From India, Karunasagar *et al*, (1990) earlier reported that *V. parahaemolyticus* is the commonest among potentially pathogenic vibrios encountered in seafoods. Even in this study, over 60% of the samples were positive for *V. parahaemolyticus* and 24% positive for *V. vulnificus* (Table 5). All the isolates of *V. parahaemolyticus* were negative for Kanagawa haemolysin production. Though it was earlier believed that only Kanagawa haemolysin producing strains are involved in gastroenteritis (Sakazaki *et al*, 1968), interest in Kanagawa negative *V. parahaemolyticus* has increased recently because Honda *et al*, (1987) reported outbreaks of gastroenteritis due to such strains. However, since this organism is native to the marine environment, it would be unrealistic to have zero tolerance for these in raw seafoods. Adequate cooking should eliminate any possibility of these organisms being a public health hazard.

V. vulnificus has also been reported to occur in coastal environments in many countries (Colwell, 1984 and Karunasagar and Karunasagar, 1991) and therefore its presence in freshly harvested shrimp is not surprising. However, this organism has been associated with septicaemia with a high mortality rate in immunocompromised persons or those with underlying hepatic disorders (Colwell, 1984). Since the organism can be easily killed by cooking, seafood consumers should be advised to eat only cooked seafood.

As shown in Table 3, sediments from shrimp harvesting areas showed bacterial counts ranging from 10^5-10^7/g. Faecal coliforms were also present in a majority of samples and, as shown in Table 4, *Klebsiella* and *Enterobacter* were the predominant organisms present. It can therefore be speculated that some members of the Enterobacteriaceae particularly, *Klebsiella* and *Enterobacter* can colonise the marine sediment and contaminate the shrimps. These might account for positive faecal coliform test in freshly harvested shrimps. Potentially pathogenic vibrios are present in shrimps and their environment and therefore it would not be practical to have a zero tolerance for these raw shrimps.

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Table 1 Bacteriological counts in a few representative samples of freshly caught shrimps

No.	TPC/g	Faecal coliform (MPN/100g)	Presence of			
			V. cholerae	*V. parahaemolyticus*	*V. vulnificus*	*Salmonella*
1	1.83 x 10^5	<2	-	-	-	-
2	6.43 x 10^5	>2400	-	+	+	-
3	3.26 x 10^5	>2400	-	+	+	-
6	7.30 x 10^4	140	-	+	-	-
7	1.98 x 10^5	130	-	-	+	-
8	4.90 x 10^5	>2400	-	-	+	-
9	2.93 x 10^5	280	-	+	-	-
11	4.65 x 10^4	-	-	+	-	-
14	2.95 x 10^6	-	-	-	-	-
16	1.94 x 10^6	24	-	-	+	-
20	2.39 x 10^6	7.9	-	+	-	-
24	1.59 x 10^6	24	-	-	-	-
30	6.25 x 10^5	4.6	-	+	-	-
32	6.85 x 10^5	0.78	-	+	+	-
34	2.37 x 10^5	3.3	-	-	-	-

Table 2 Incidence of various Enterobacteriaceae members in samples of freshly caught shrimps testing positive for faecal coliforms

Sample No.	Presence of			
	E. coli	*Klebsiella*	*Enterobacter*	*Citrobacter*
2	+	+	+	-
3	-	+	+	-
6	-	+	-	-
7	-	+	-	-
8	-	+	-	-
9	-	+	+	-
16	-	+	+	-
24	-	+	-	+
26	-	+	-	-
28	-	+	+	-
29	-	-	+	-
30	-	+	-	-
32	-	+	+	-
34	-	+	+	-

Table 3 Bacteriology of a few representative samples of sediments from shrimp harvesting areas

Sample No.	TPC/g	Faecal coliform (MPN/100g)	Presence of			
			V. cholerae	*V. parahaemolyticus*	*V. vulnificus*	*Salmonella*
1	6.35×10^5	-	-	+	-	-
2	1.22×10^6	2.3	-	+	+	-
3	3.65×10^6	0.2	-	-	+	-
6	2.27×10^6	-	-	+	-	-
8	9.35×10^5	24	-	+	-	-
9	3.15×10^6	13	-	-	+	-
10	1.56×10^6	79	-	+	-	-
14	1.20×10^7	24	-	-	+	-
16	1.54×10^6	78	-	+	+	-
19	1.78×10^6	24	-	+	-	-
22	1.30×10^5	8	-	-	-	-
26	1.80×10^6	42	-	+	+	-
30	1.17×10^7	84	-	-	+	-
34	1.34×10^6	-	-	+	+	-
38	1.54×10^6	110	-	+	-	-

Table 4 Incidence of various Enterobacteriaceae members in samples of sediments testing postive for faecal coliforms testing positive for faecal coliforms

Sample No.	Presence of			
	E. coli	*Klebsiella*	*Enterobacter*	*Citrobacter*
1	-	+	-	-
2	-	+	+	-
3	-	+	-	-
8	-	-	+	-
9	-	+	-	-
10	-	-	-	+
14	-	+	-	-
16	-	-	+	+
19	-	+	-	-
22	-	-	+	-
26	-	-	+	-
30	-	+	-	-
38	-	-	+	-
		+		-

Table 5 Percentage incidence of pathogenic vibrios

Sample	Incidence of	
	V. parahaemolyticus	*V. vulnificus*
Shrimps	62	24
Sediment	51	18

SALMONELLA AND *VIBRIO CHOLERAE* IN CULTURED TROPICAL PRAWNS

by

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ABSTRACT

The occurrence of *Salmonella* and *Vibrio cholerae* in brackishwater ponds was monitored over a two year period in one of the major prawn exporting countries in Southeast Asia. The principal production areas were identified and regular samples taken for *Salmonella* and *V.cholerae* analysis. Results demonstrated that brackishwater ponds and cultured prawns were inherently contaminated with both bacterial pathogens. *Salmonella* spp. were present in 16% of prawns and 22% of mud/water samples from ponds and *V.cholerae* present in 1.5% of prawns and 3.1% of mud/water samples. Culturing by intensive methods tended to favour contamination by these pathogens, which is most likely due to the accumulation of waste and increase in the volume of sediments in ponds. Typical environmental factors such as water temperature, pH, and salinity were all favourable for growth of microorganisms. The incidence of the pathogens increased during the wet season and was marginally higher when ponds were located close to urban areas. *Salmonella weltevreden* was identified as the principal serotype found in ponds, and to a lesser extent *S.anatum (11%) S.wandsworth* (8%) and *S.potsdam* (8%). The *V.cholerae* belonged to the non-01 serogroup. The implications of these findings for export markets and the prawn processing industry are discussed.

INTRODUCTION

At the previous meeting of the Indo Pacific Fisheries Commission Working Party on Fish Processing and Marketing, Bangkok, Thailand in 1988, it was concluded that there was insufficient evidence available to suggest that *Salmonella* and *Vibrio cholerae* were natural contaminants of pond cultured shrimp from the Asian region. The collection of data on the incidence of these pathogens in aquaculture products was recommended as a priority research topic for workers in the region.

In 1989, over 8,300 tonnes of frozen raw prawns were detained by the USFDA due to contamination with *Salmonella sp.* (USFDA, 1989). The detention at port of entry can lead to additional costs of shipping, cold storage, handling and processing which are all charged to the exporter. It can also lead to total rejection or re-exportation of shipments, which results in severe economic losses. Exporters can insure against rejection, but costs are high at 7% of shipment value. Similar problems, although on a smaller scale, are faced by exporters to the Japanese market. The Japanese will accept *Salmonella* in raw frozen produce but will reject all products which contain *Vibrio cholerae.* Data on recent rejections are not available, but between 1983 and 1986 prawns valued at 3 million US dollars from South East Asia were rejected and destroyed by health authorities in Japan due to the presence of pathogenic *V. cholerae* (Reilly, 1987). The rationale for the USFDA standards for absence of *Salmonella* in raw frozen prawns is based on the fact that prawns of marine origin do not naturally harbour this group of bacteria and that any contamination is likely to be of human origin as a result of poor sanitation and standards of hygiene during processing. Some exporting countries argue that *Salmonella* may be naturally present in pond cultured prawns and there may be a case for revising standards.

The Natural Resources Institute (NRI) in collaboration with one of the main exporting countries of cultured shrimp in South East Asia initiated a two year survey on the incidence of *Salmonella* and *Vibrio cholerae* in brackishwater ponds and in the cultured products. The objective of this work was to survey the incidence of *Salmonella* and *V. cholerae* in brackishwater ponds, to identify the influence of environmental factors such as pond location, weather conditions, pond growth practices and water quality on the occurrence of these microorganisms.

METHODOLOGY

A total of 304 samples of prawns and mud/water samples were taken from 131 ponds in major aquaculture regions between February 1988 and January 1990. Samples were taken throughout the harvesting period in order to obtain, a representative sample of the total pond population. The size of the sample depended upon the pond area and the quantity of prawns harvested. Approximately 1 kilogram of prawns was taken when the harvest size was about 2.5 tonnes per hectare. Composite sub-samples of 100g were taken for bacteriological analysis by aseptically sampling 2-3g from individual prawns. The samples were taken randomly and included part of the shell, gut, flesh, and head of prawns. Representative samples of water and mud from the bottom of ponds were taken for analysis, which consisted of a 1 litre composite sample from at least five different locations in the ponds. Samples contained approximately 1:1 mud:water.

Samples were analyzed for *Salmonella* and *V. cholerae* using the standard methods recommended by the USFDA (1978). The identification of suspect colonies was confirmed at the NRI, and isolates were serotyped by the Public Health Laboratory Service, UK. In order to obtain a representative cross-section of the brackishwater-aquaculture industry, samples were collected from ponds which used extensive, semi-intensive and intensive culturing systems. Various environmental factors were also monitored during the course of this survey and these factors were correlated with the presence/absence of both pathogenic bacteria. The factors included the type of feeds used in ponds, data on wet and dry seasons, pond location, pond reconditioning, water salinity, temperature and oxygen concentration.

RESULTS AND DISCUSSIONS

Results from this survey showed that *Salmonella* spp. were present in 16% of prawns and 22% of mud/water samples and that *V. cholerae* was present in 1.5% of prawns and 3.1% of mud/water samples from ponds (Table 1). These results clearly demonstrate that *Salmonella* is present in brackishwater ponds and that it can also be found as part of the natural microflora of cultured prawns. Import standards for raw frozen prawns for the USA are based on the fact that *Salmonella* species are not naturally present in the marine environment and therefore are unlikely to be detected in freshly harvested produce. Additionally *Salmonella* in raw frozen prawns are thought to be due to unhygienic handling and poor manufacturing practices. Results from this survey indicate that bacteria present on cultured prawns are very different to those reported in the marine species and that the bacterial flora of prawns is a reflection of the environment.

The occurrence of both *Salmonella and V. cholerae* in the environment is a serious cause for concern for both processors and exporters. There is a high risk that contaminated prawns will be accepted into processing plans and that pathogens will be detected in exported raw frozen products. Data from Table 1 shows this to be the case. There is also an additional risk that cross-contamination between raw and cooked products will occur in processing plants, particularly when processors are unaware that products are likely to be naturally contaminated with both pathogens. Public health authorities in the UK have found the same serotypes of *Salmonella* as have been isolated from ponds during this survey in cooked prawns from Thailand and Malaysia. Prawn processors, particularly those involved in the preparation of value added products, must be made aware of the high risk involved with processing brackishwater cultured species for export.

The usual method of handling cultured prawns after harvesting is chilling in ice water followed by inspection/grading/weighing and icing. These post-harvest practices will not remove either *Salmonella* or *V. cholerae* if present on the prawns. It may be possible to wash prawns at the pond site, if good quality potable water is available, but this additional step will not guarantee the removal of either pathogen. Washing prawns will reduce surface bacteria numbers by about 90%, and the addition of chlorine to water for washing may remove an even higher number, but will not necessarily yield a pathogen free product. When *Salmonella* or *V. cholerae* are present in the gut of prawns, a washing step is unlikely to be effective. During the course of this survey 18 gut samples were tested for the presence of both pathogens in order to identify if either were established in the digestive systems of prawns. Although all samples were negative, data from one of the major exporters of prawns on a larger number of samples have shown that *Salmonella* can be present in the gut of prawns.

Table 1 Incidence of pathogenic bacteria in prawns and pond (mud/water) samples from major production areas

Region	No. of ponds surveyed 1988/90	Number of samples where:			
		Salmonella present		*V. cholerae* present	
		Prawns	Mud/water	Prawns	Mud/water
A	11	2	4	0	0
B	25	6	7	1	3
C	29	2	5	0	0
	7	0	2	0	0
D	39	7	8	1	1
E	8	1	1	0	0
F	12	3	2	0	0
TOTAL	131	21 (16%)	29 (22%)	2 (22%)	4 (3.1%)

Table 2 shows the *Salmonella* isolates identified to species levels. In total, 11 different serotypes were isolated, some of which are well known human pathogens. *S. weltevreden* was the most common species isolated and this organism has been previously reported as a common contaminant of freshly harvested cultured fish (Iyer and Shrivastava, 1989). It must be emphasised that the normal cooking process of prawns will destroy all the *Salmonella* species isolated. D-values for *S. weltevreden* in a brown shrimp homogenate have been reported to be 0.68, 0.61 and 0.38 minutes at 60, 61.4 and 62.8°C respectively (McPherson and Zywno, 1985). Between 1980 and 1982 approximately 200 Salmonellae isolates per year were recovered by the USFDA, and the most frequently isolated serovar has been *S. weltevreden* (McPherson and Zywno, 1985). Although data on the serotypes of *Salmonella* isolated by the USFDA are not available, our results suggest that the contamination is coming from ponds and not from poor standards of hygiene and processing.

The occurrence of pathogens in aquaculture products has been extensively reported. A survey in Japan has shown that *Salmonella* species were present in 21% of eel culture ponds (Saheki *et al.*, 1989). In the USA freshwater cultured catfish have been found to harbour *Salmonella* species with an overall incidence rate of about 5% (Wyatt et al., 1979). Data from the previous IPFC Working Party on Fish Marketing and Utilisation in 1988 suggested that brackishwater ponds in the South East Asian region contain *Salmonella.* Aquatic birds are known to harbour pathogenic strains of *V. cholerae* and *Salmonella,* and have been suggested as one possible means of spreading this organism from area to area (Ogg *et al.*, 1989; Fenlon, 1983).

Table 2 Sero types of *Salmonella* isolated from prawns and mud/water samples

Serotype	% isolation
S. weltevreden	47
S. anatum	11
S. wandsworth	8
S. potsdam	8
S. newport	5
S. abony	5
S. lansing	3
S. thomson	3
S. houten	3
S. brunei	3
S. hvittingfoss	3

In an attempt to identify the sources of pathogens in ponds, various factors were monitored and correlated with positive results for both *Salmonella* and *V. cholerae*. Table 3 shows the relationship between the type of feeds and the occurrence of both pathogens. Natural feeding relies on the growth of algae and plankton on which prawns feed and is generally used in extensive culture systems. Fertilisers are employed during pond preparation and recommendations by the South East Asian Fisheries Development Centre are for the application of one tonne per hectare of chicken manure to ponds (SEAFDEC, 1989). This may be one source of pathogens in the ponds. Supplemental feeding involves the addition of small fish (dried or raw) and crushed shellfish to ponds in addition to other feeds. Most ponds surveyed use commercial feeds either exclusively or in combination with other feeds. Table 3 shows that the highest incidences of both *Salmonella* and *V. cholerae* were found in ponds using commercial feeds. It is very unlikely that these feeds are the source of pathogens because of the high temperature involved in their production and the repeated bacteriological analysis which have shown them to be free of contamination (Reilly, unpublished results). A more likely explanation is that when feeding rates are high, residual or waste feeds will accumulate in ponds. This will contribute to a high organic loading in ponds which in turn will favour an increase in the number of microorganisms, such as *Salmonella* and *V. cholerae.* Other factors such as faeces from aquatic birds, prawn excrete, and possibly that from farm animals and land drainage will all contribute to the organic loading of ponds. It is unlikely that this problem can be overcome without redesigning brackishwater ponds and changing firmly established aquaculture practices.

Table 3 Relationship between prawn feeds and the incidence of bacterial pathogens

Feed type	No. of ponds	Incidence of:			
		Salmonella		*V. cholerae*	
		Prawn	Mud/Water	Prawn	Mud/Water
Commercial	98	18	23	2	4
Natural	12	0	2	0	0
Natural/commercial	4	1	1	0	0
Commercial/supplemental	5	0	1	0	0
Natural/supplemental	12	2	2	0	0

The relationship between culture methods and the incidence of both pathogens is shown in Table 4. In the extensive system ponds are stocked with about 10 fry per square metre. Aeration is not used and prawns feed on naturally occurring organisms. The net result of this culture method is that yields are low and grow-out periods extended. The average yields observed in this survey were in the order of 400-600 kg per hectare over grow-out times of 6-8 months. In the semi-intensive systems fry were stocked in the range of about 10-17 per square metre. Average yields were 2-3 tonnes per hectare per harvest and about 2 harvests per year were achieved. Stocking densities were increased to 18-30 fry per square metre in intensive systems. Feeding rates and aeration were also increased and yields of about 6 tonnes per hectare per harvest were attained. The extensive system can be regarded as a form of polyculture as other species grow and are commercially harvested. Both semi-intensive and intensive methods are monoculture systems where pisicides are employed to remove competing species. The highest incidence of *Salmonella* in both prawns (25.5%) and mud/water (31.4%) from ponds occurred when intensive culture systems were used. The isolation of *V. cholerae* was also highest with intensive systems. These observations are consistent with those regarding high organic loadings encouraging the growth of microorganisms. Accumulation of feed and sediments in ponds will lead to loss in water quality. Excessively high stocking densities will subject the prawns to stress and result in inferior quality products.

Table 4 Relationship between culture methods and incidence of bacterial pathogens

Culture methods	No. of ponds	Incidence of:			
		Salmonella		*V. cholerae*	
		Prawns	Mud/water	Prawns	Mud/water
Extensive	26	1	5	0	0
Semi-intensive	56	6	7	1	1
Intensive	51	16	17	1	3

Table 5 shows the effects of different environmental factors on the incidence of both pathogens. A higher incidence was observed during the wet season, which is probably due to an increase in drainage to coastal areas and rivers, which are the sources of water in ponds. Direct land drainage into ponds is also a possibility. During the wet season *Salmonella* was isolated from prawns in 20% of the samples, as compared to 13.5% for the dry season. *V. cholerae* was not isolated from prawns during the dry season, only during the wet season.

The location of ponds sampled during the survey was recorded in order to study if there was a relationship between the distance of ponds from farms, houses. villages, etc., and contamination by pathogens. There was a slightly higher incidence of Salmonella in ponds which were located in urban and semi-urban areas (Table 5). The highest occurrence of pathogens in ponds was recorded in the most densely populated area of the country where the survey was carried out.

The length of time between harvesting and restocking ponds is referred to as pond reconditioning time. Inadequate reconditioning of ponds was one of the reasons for the failure of the prawn aquaculture industry in Taiwan during 1988/89. Table 5 relates pond reconditioning time to the isolation of both pathogens, but the data is inconclusive.

Table 5 Effects of environmental factors on the incidence of bacterial pathogens

Environmental factors	No. of ponds	Incidence of:			
		Salmonella		*V. cholerae*	
		Prawns	Mud/water	Prawns	Mud/water
Weather:					
Dry season	81	11	16	0	2
Wet season	50	19	13	2	2
Location of Ponds					
Urban					
Rural	51	6	12	1	3
Semi-rural	11	3	2	1	1
	69	12	15	0	0
Pond reconditioning time					
1-4 weeks	63	10	10	1	2
5-6 weeks	18	3	5	0	1
7-8 weeks	2	0	0	0	0
Over 8 weeks	12	1	6	0	0
Not monitored	36	7	8	1	1

CONCLUSIONS

The results presented here indicate that both *Salmonella* and *V. cholerae* are present as part of the natural flora of brackishwater cultured prawns, and these findings are a serious cause for concern for both processors and exporters. Prawn processors are generally unaware that cultured products may harbour both *Salmonella* and *V. cholerae* and of the high risks of cross-contamination between these and raw marine shrimp or other processed products. Raw brackishwater cultured prawns should be placed in a different public health risk category to marine species. Alternatively, raw brackishwater cultured shrimp could be classified in the same classification system as whole raw chicken, which usually contains *Salmonella.*

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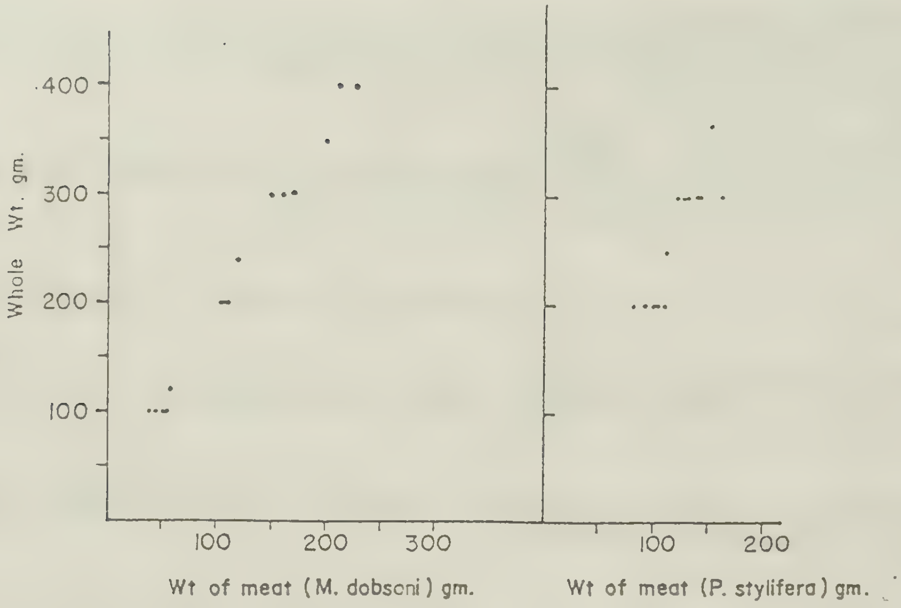

Fig. 1. Plot of meat recovered against the quantity of whole prawns taken.

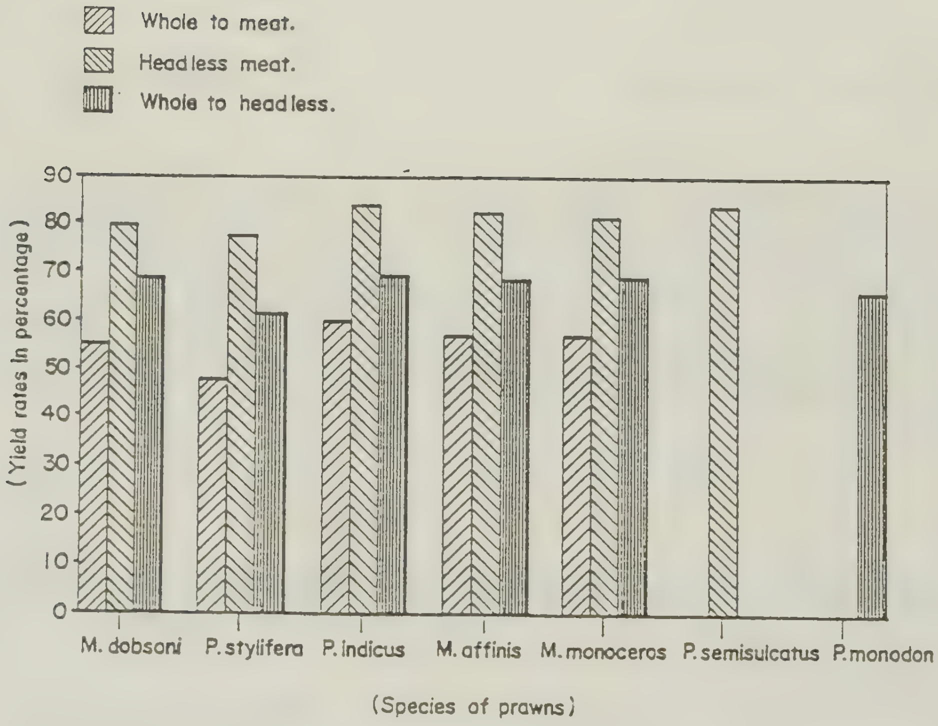

FIG. 2. YIELD RATES IN PERCENT

QUALITY OF CULTURED TIGER PRAWN (*Penaeus monodon*) AND DETERIORATION DURING STORAGE: A REVIEW

by

Rowmawaty Peranginangin
Suparno, and Ijah Mulyanah

ABSTRACT

Environmental quality of brackish water ponds for tiger prawn (*Penaeus monodon*) culture from Cemara village influence the quality of final products. The highest mercury (Hg) content of the products was 0.375 ppm, which is lower than the maximum tolerance limit of 0.5 ppm, (indicate that the culture area has not been polluted yet by that heavy metal). The source of mercury was not known since water did not show any sign of pollution; however, sediment contained a relatively high mercury content (the highest level was 0.5 ppm). Pathogenic bacteria and *E. Coli* were also not detected. However, total bacterial counts and Staphylococcal counts were considerably high due apparently to poor handling. Treatment of prawn by dipping in solution of $Na_2S_2O_6$ 3000 ppm and NaOC1 100 ppm for 10 minutes was able to minimize the total bacterial counts, Staphylococcal and Coliform on whole and headless prawn held at ambient temperature, icing and frozen storage. Dipping in the solution of $Na_2S_2O_6$ 3000 ppm for 10 minutes was also able to prevent formation of black spots in the prawn.

INTRODUCTION

Prawn is the main fishery export commodity from Indonesia. Historically, most of the prawn produced was harvested from natural beds but in order to boost productivity, culture of tiger prawn (*Penaeus monodon)*, is being extended (Anonymous, 1983).

There are problems with the quality of the product. According to Ruello (1976), importing countries usually impose strict (chemical and microbiological) standards of quality. Any product which does not meet the standards will be rejected. Generally the reason for rejection of the product of Indonesia by USFDA is poor hygienic condition, i.e. contamination of pathogenic bacteria, especially *Salmonella sp*. and bacteria of faecal origin *(E. coli)* as well as physical impurities (Suyanto, 1984; Putro, 1984). The important factors affecting the quality of the product are the environmental conditions of the brackish water ponds in which the prawn is cultured, temperature, handling and processing methods. The present review describes experiences from work conducted at the Research Station for Marine Fisheries Slipi, Jakarta.

ENVIRONMENTAL CONDITION OF CULTURED PRAWN (*Penaeus monodon*)

Environmental conditions of the brackish water ponds for prawn culture affect the quality of the product. The limited area available for culturing, the high density of the population around the area, and the rapid industrial growth (especially in Java) contribute negatively to the environment. With this regard Sarnianto *et al* (1985) have studied the quality of tiger prawn and its environment (water and sediment) collected from five ponds of Cemara village, Karawang, West Java. They found the mercury (Hg) was not detected in water from the five pounds but mercury contents are in the range 0.077-0.508 and 0.189-0.375 for sediment and prawn respectively. The maximum tolerable concentration recommended by WHO is 0.5 ppm. This suggests that the prawns were safe for consumption. Based on an acceptable daily intake (ADI) for man of 0.05 ug per kg of body weight (FAO/WHO, 1967 in Keckes and Miettinen, 1976), up to 6.67g of flesh daily or 46.67g weekly for man with body weight of 50 kg could be safely consumed. It is interesting to note that, although mercury was not detected in the water, considerable levels were found in prawn and sediment. The source of mercury is still not clearly known. Occasional mercury contamination from water, natural presence of mercury in soil of the ponds, and other materials from farming operation (e.g. feeds, fertilizer, insecticide) are possible sources.

Total bacterial counts (TPC) were in the range 0.2 x 10^5 per gram. Compared to the Indonesian standard for frozen prawn x 10^5 per gram, the microbial load of prawn is substantial in some samples. High bacterial loads imply that the products need special handling and processing to reduce the bacterial load.

The higher the bacterial load of the habitat (i.e. water and sediment) the higher the bacterial load found in the prawn. There is a relationship between the TPC and Staphylococcal counts of prawn and their habitat. In all samples, except for samples from pond 2, *Salmonella sp.* was not detected. The low content of coliforms and faecal coli of the samples (water, sediments and prawn) indicate that the prawn cultures area of Cemara village has not been microbially polluted yet.

DETERIORATION OF PRAWN (*Penaeus monodon*) AT AMBIENT TEMPERATURE

An observation carried out by Ilyas and Nasran (1972) on whole prawn from Cilincing pond (Jakarta Bay) has shown the initial TPC level was 7.6 x 10^4 but increased after 6 hours to 2.5 x 10^6 at ambient temperatures of 28.5 x 32.2°C. At temperatures between 24 to 28.5°C shrimp was rejected by the panel after 8 hours storage while at temperatures of 28.5 to 32.2°C shrimp was rejected after 6 hours with a total bacterial load 3.6 x 10^6 cells/g and 2.5 x 45.9 mgN% and at triethylamine (TMA) levels of 1.1-5-7 mgN%.

To extend keeping quality of whole and headless prawn Murniyati *et al* (1985) used NaOC1 solution. Dipping the whole prawn at a concentration of 100 ppm for 10 minutes and headless prawn at a concentration of 50 ppm for 10 minutes reduces bacterial count from log 5.5 to log 5.0 for whole prawn, and log 5.5 to log 4.8 for headless prawn. NaOCl solution also reduced staphylococcus counts, coliform and H_2S producing bacteria when prawns were dipped in 100 ppm for 10 minutes for whole prawn and 75 ppm for 10 minutes for headless prawn. Blackspot is one criteria for evaluation of prawn quality. Blackspots, formed through enzymatic processes, develop rapidly after the prawn dies. To prevent blackspot formation Peranginangin *et al* (1985) carried out an experiment of dipping prawn in 3000 ppm solution $Na_2S_2O_6$ solution for 10 minutes. The percentage of blackspot formed after 7 hours of delayed icing were 80-100% and 30-60% for whole and headless prawn respectively. Dipping prawn in $Na_2S_2O_6$ solution also reduced total bacterial counts from log 5.9 to log 4.6 for whole prawn and from log 5.5 to log 4.4 for headless prawn. H_2S producing bacteria decreased from log 4.3 to log 2.7 for whole prawn and from log 3.0 to an undetectable level for headless prawn. Coliforms and Staphylococcus also decreased to 9 MPN/g and log 3.1 respectively.

EFFECTS OF CHILLING ON DETERIORATION *(Penaeus monodon)*

Frozen shrimp is the most important commodity for export, and needs proper handling to maintain the quality from immediately after capture until freezing in the processing plant. The effects of chilling methods on deterioration of prawn before they were frozen have been studied. Tolerable limits for handling of prawn by various chilling methods have been set up. Prawn held in chilled sea water should be frozen within 5.7 days with pH 7.1; TVB 25.32 mgN% and TPC log 5.33 as the quality parameter levels. Shrimp held in chilled fresh water should be frozen within 4.5 days with pH 7.01; TVB 23.37 mgN% and TPC log 5.65 and should be frozen within 5.57 days (Soedijono *et al*, 1986).

Anggawati *et al* (1986) has studied use of chlorine solution and $Na_2S_2O_6$ solution to reduce total bacterial count and to prevent blackspot formation on whole prawn and headless prawn. Blackspot formation was prevented for 4 days on whole prawn and 8 days on headless prawn (*Penaeus monodon*) when dipped in a 3000 ppm of $Na_2S_2O_6$ solution for 10 minutes before icing. (Figure 1).

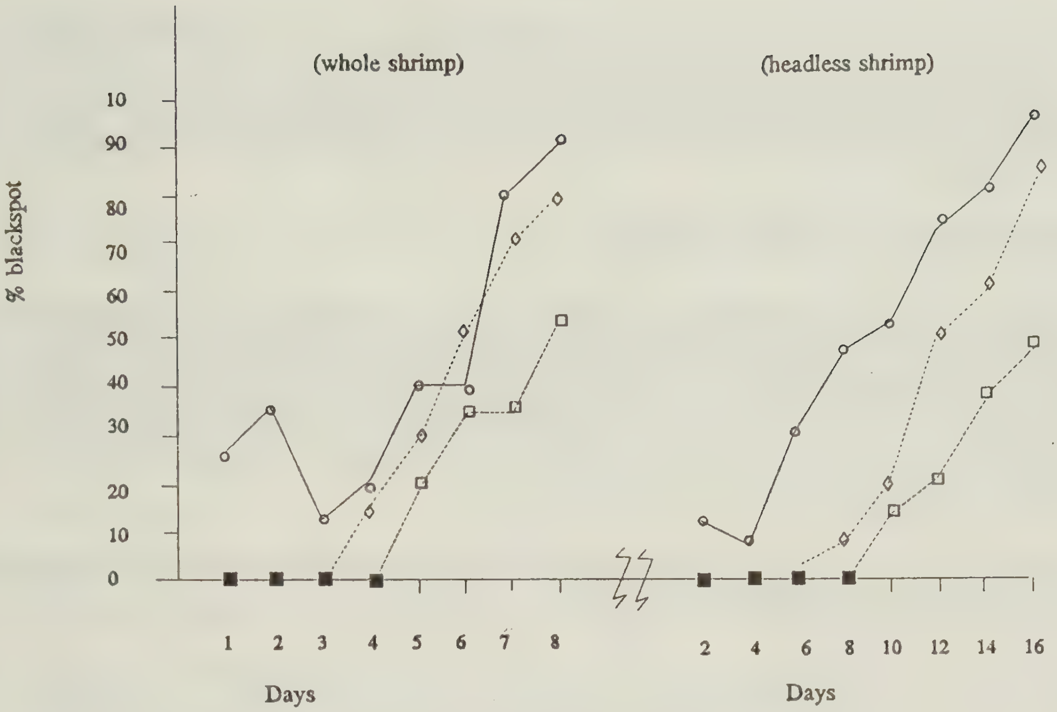

Figure 1 Percentage of shrimp developing blackspots during storage in ice
(o–o dipped in tap water, □–□ dipped in $Na_2S_2O_6$, △...△ dipped in NaOCl

Chlorine solution proved to be a good bactericidal agent for reducing the microbial populatial on shrimp. Shrimp dipped in 100 ppm chlorine solution for 10 minutes before icing had a lower bacterial count in comparison to those dipped in tap water (Figure 2).

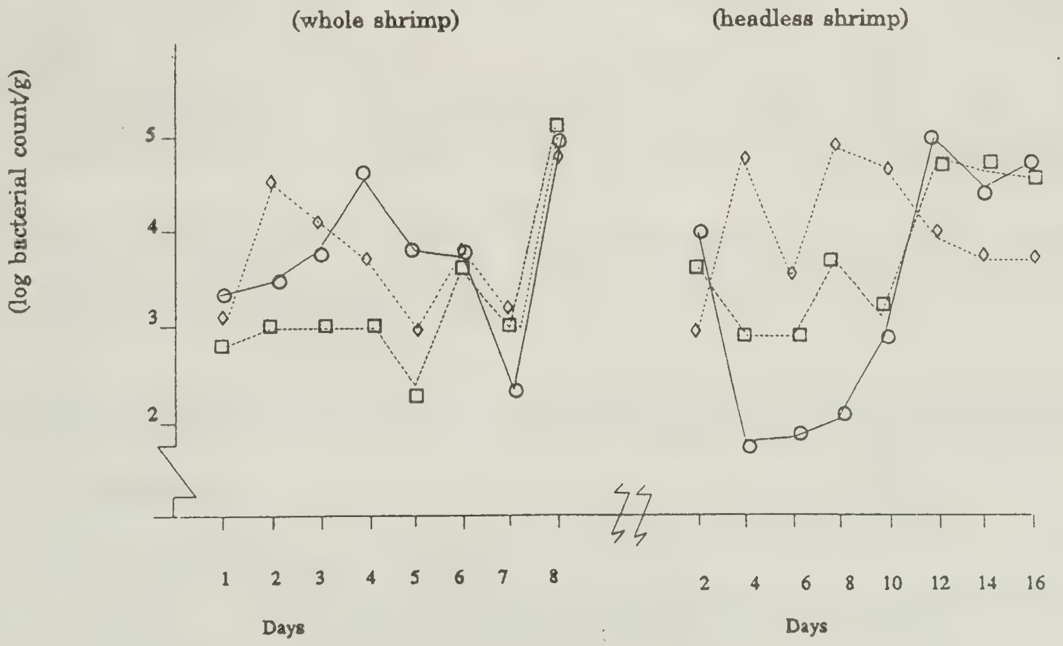

Figure 2 Changes in the total bacterial count of shrimp
(o–o dipped in tap water, □–□ dipped in $Na_2S_2O_6$, △...△ dipped in NaOCl

Organoleptic tests showed that the changes of appearance, odour and texture were observed by panellists after 5 days for whole prawn and 9 days for headless prawn. The effect of dipping in $Na_2S_2O_6$ and NaOC1 solutions appears to be reduced after 4 days storage for whole prawn and 8 days for headless prawn. According to Ruello (1976) in Australia the use of sulphite solution should not result in residues higher than 30 ppm. The United States Food and Drug Administration (USFDA) enforces a limit of residual sulphite of 100 ppm.

PRAWN QUALITY IN FROZEN STORAGE

The effects of sodium metabisulfite and sodium hypochlorite treatment on the quality of prawn have also been studied by Murniyati *et al* (1991). Whole and headless prawn were dipped in a solution of 3000 ppm $Na_2S_2O_6$ and 100 ppm NaOCl for 10 minutes before freezing. Monthly analysis of organoleptic, chemical and microbiological properties were performed during 5 months frozen storage at -20°C. Blackspot occurred on whole and headless prawn to the extent of 20% and 10% respectively after 4-5 months storage. Dipping in sodium meta-bisulphate and sodium hypochlorite solution prevented development of undesirable odours until 5 months storage.

The sensory evaluation showed that headless prawn dipped in sodium meta bisulphate solution had the highest score and the lowest bacterial count.

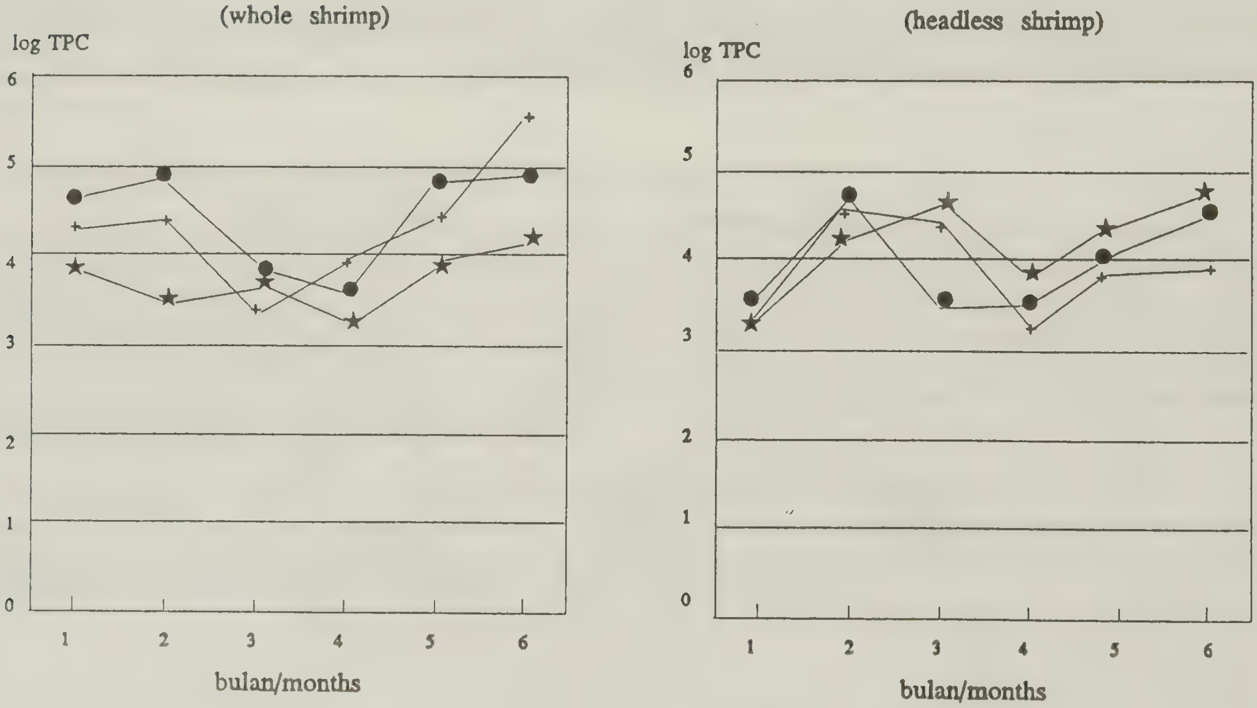

Figure 3 Total Plate Count changes of shrimps dipped in tap water. NaOCl and $Na_2S_2O_6$ solution during frozen storage

CONCLUSION

1. Post harvest quality of cultured tiger prawn is affected by various factors namely environmental conditions (water, soil/sediment), farming operation (feed, fertilizer, insecticide), handling and processing.

2. Initial bacterial loads are generally high in cultured prawn due mainly to high bacterial load of the pond environment.

3. Disinfection of raw material for prawn processing appears to be essential because of high initial bacterial loads. Sodium bisulphate and chlorine have been proved to be capable of reducing the bacterial loads, and thus prolong shelf life of stored prawn. Care should be taken in using chemical in order to restrict residual levels in the product.

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Table 1. Chemical and microbiological evaluation of whole fresh prawn, water and sediment from brackish water ponds of Cemara village

Ponds	Staphylo 10^2	Salmo-nella	Coliform MPN	Faecal coli MPN	TPC 10^6	TMA mg%N	TVB mg%N	pH	Hg (ppm)
Water									
1	0.2	nd	210	nd	79-219				nd
2	23.4	nd	1100	nd	296-756				nd
3	nd	nd	nd	nd	0.2-0.3				nd
4	2.8	nd	9.1	nd	4-17				nd
5	nd	nd	3.6	nd	0.3-0.9				nd
Sediment									
1	80-166	nd	160	100	608-658				0.210
2	2520-5400	43	1100	200	1792-2228				0.244
3	63-68	nd	34	10	4-53				0.077
4	189-208	nd	290	100	648-790				0.245
5	68-71	nd	120	100	55-90				0.508
Prawn									
1	210-280	nd	1100	nd	3.4-4.7	0.18-0.74	14.11-15.97	6.65	0.205-0.233
2	1610-2300	nd	1100	nd	5.2-23.3	0.38-1.11	15.60-18.99	6.75	0.249-0.375
3	30-90	nd	460-1100	nd	0.2-0.7	1.11-1.48	20.06-20.80	6.87	0.189-0.205
4	970-1260	nd	1100	nd	16.2-36.8	0.55-1.11	17.83-18.57	6.60	0.203-0.220
5	110-390	nd	1100		6.6-12.6	0.37-1.30	13.00-14.86	6.57	0.260-0.344

THE COMBINED EFFECT OF CHLORINE AND FROZEN STORAGE ON MICROBIOLOGICAL QUALITY OF CULTURED SHRIMP (*Penaeus monodon*)

by

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ABSTRACT

The effect of chlorine and frozen storage on the microbiological quality of cultured shrimps was investigated. It was found that soaking the shrimp in 10 ppm chlorine reduced specific growth rate (SGR) of TCP, Coliform, *E. coli* and *Salmonella*. However, storage time at -20°C, qualitative analysis gave positive results.

INTRODUCTION

Most of the shrimp exported from Indonesia are packed in frozen blocks. The environment of the pond has a direct influence on the product quality as well as does period of handling and processing, which inevitably increases microbiological quality by contamination from hands and equipment used (Sunarya, 1991). This can be reduced by washing the shrimp with good quality water or disinfectants such as chlorine. Sunarya (1991) also described increasing "total plate count" in cultured shrimp on harvesting, handling and processing. During transportation to the processing unit counts go up a bit especially during sorting, beheading and arranging in the pan before freezing. Increasing total plate count during handling and processing may also involve some pathogenic microorganisms which will be reduced after freezing (Muctaroni *et al.*, 1990 and Haryanto, 1990).

For these reasons, a study on the effect of chlorine and frozen storage on reducing the numbers of some microorganisms has been conducted.

MATERIALS AND METHODS

Cultured shrimp *(Penaeus monodon)* were collected from the processing units of Pumar Ltd. and Halimas Sakti Ltd. Jakarta. The shrimp were very fresh because they had just arrived from the pond. They were transported in a clean styrofoam box with ice to the National Centre for Fish Quality Control and Processing Technology Development (NCQC), Jakarta, in approximately five minutes.

At the laboratory of NCQC, the shrimp was washed with clean water and beheaded. The beheaded shrimp were then divided into two groups - group 1 was put in 10 ppm chlorine solution for 30 minutes and group 2 was put in clean water. Both groups 1 and 2 were then put into polyethylene bags and frozen in an air blast freezer at -40°C for 4 hours. They were then stored at -20° until analyzed.

For coliforms (include *E. coli*) and *Salmonella* evaluation, similar procedures were carried out, but the beheaded shrimp was inoculated with *Coli* and *Salmonella* respectively before treatment with clean water or 10 ppm of chlorine solution. Inoculation of *E. coli* and *Salmonella* were done by soaking in the *E. coli* suspension or *Salmonella* suspension, in the refrigerator overnight (Sunarya *et al.*, 1989). The suspensions contained 6.66×10^{10} *E. coli* or *Salmonella* per litre and the ratio of shrimp and solution was 1:4.

Microbiological analysis of the frozen samples was done after freezing (0 storage), after two weeks storage and after four weeks storage.

Total plate count was analyzed using methods developed by Messer *et al.,* 1984 for shrimp samples (without any inoculation of *E. coli* or *Salmonella*.

MPN of coliform and MPN of *E. coli* were analyzed using methods developed by Mehlman *et al.,* (1984) for *E. coli* inoculated shrimp samples.

Quantitative analysis of *Salmonella* was carried out using plate XLD selective agar. Before plating, enrichment of *Salmonella* was done on TTB and BFP medias.

Qualitative analysis for *Salmonella* was done using BAM methods (Andrews *et al.,* 1984).

Data were evaluated by calculation of "specific growth rate" (SGR) calculated from the log of microorganism numbers at the time of storage, divided by log of microorganism numbers of the control (0 time storage for sample without any treatment with chlorine).

RESULTS AND DISCUSSION

The specific growth rates (SGR) for total plate count (TPC) are tabulated as Table 1.

Table 1. Total plate count and SGR of sample during storage at -20°C

Sample	storage time (week) at -20°C	TPC	SGR
untreated	0	3.18×10^6	1.00
	2	8.07×10^5	0.91
	4	2.24×10^5	0.82
treated with chlorine	0	1.79×10^6	0.96
	2	5.00×10^5	0.88
	4	1.12×10^5	0.78

Table 1 shows that SGR for all of the samples decreased especially samples with chlorine. Storage time also correlates with the degree of decreasing SGR where the longer storage time gave lower SGR. Similar results were also observed for the SGR of Coliforms, *E. coli* and *Salmonella* as tabulated in Table 2.

Table 2. Specific growth rate of coliform, *E. coli* and *Salmonella* of sample during storage at -20°C

sample	storage time (week) at -20°C	Coliforms		*E. coli*		*Salmonella*	
		MPN	SGR	MPN	SGR	Plate count	SGR
untreated	0	350	1.00	7,800	1.00	22,400	1.00
	2	131.5	0.83	44	0.42	295	0.74
	4	17.5	0.49	<3	0	<3	0
						11,600	0.96
treated	0	93	0.77	1,425	0.81	36	0.62
with	2	19	0.50	22	0.34	<3	0
chlorine	4	12	0.42	<3	0		

From Tables 1 and 2 it can be seen that soaking in 10 ppm chlorine solution for 30 minutes can reduce TPC, Coliforms, *E. coli* and *Salmonella* for all the samples, because chlorine is a bactericide which can decrease the activity of the glucose oxidation enzyme and other metabolic processes of the microorganisms (Jenie, 1988).

SGR at 0 time storage for Coliforms (0.77) and for *E. coli* (0.81) were lower than for TPC (0.96) probably indicates that chlorine is a more effective bactericide for gram negative microorganisms as mentioned by Rose and Kotula (1984),. Some gram positive microorganisms were possibly present in the control shrimp sample giving a higher SGR for TPC.

Table 2 also shows that the SGR for *E. coli* was higher than Coliforms at 0 storage - in fact the shrimp sample was inoculated with *E. coli*. This may indicate that the sample was already contaminated with other coliforms such as *Aeromonas hydrophila, Enterobacter Klebsiella* and *Citrobacter* (Mahlman *et al.*, 1984), which have different sensitivities to chlorine.

The same SGR (0.96) for salmonella and TPC (treated with chlorine) indicate that salmonella was not sensitive to chlorine, compared with coliform/*E. coli*. However, in the second week's storage at -20C the SGR of *Salmonella* (0.62 for sample treated with chlorine and 0.74 without treatment were lower than SGR of TPC. This indicates that time of storage at 20°C was effective for reducing *Salmonella*, a non-psychrophilic microorganism as mentioned by Desrosier and Tressler (1977). Similar results were also found for Coliforms and especially for *E. coli* in that SGR decreased during storage at -20°C. Although *Salmonella* could not be counted after 4 weeks storage at 20°C, qualitative analysis gave positive results for all the salmonella samples. This is because the quantitative analysis (using plate count) was not sensitive enough for *Salmonella*. Positive results of qualitative analysis of *Salmonella* indicate that it is difficult to kill all of the organisms under frozen storage conditions (Robinson, 1985; Troller, 1983) and the number of *Salmonella* can be reduced from 23 - 167 x 10^6/g to 0.86 - 34 x $10^{6/}$g in frozen storage at -25° for 270 days (Jay, 1978). So, in order to produce good quality frozen shrimp (free from *Salmonella*), prevention of any contamination and a decrease in the growth of salmonella during handling and processing of shrimp are needed. Improvement of hygienic conditions and application of GMP for handling and processing of shrimp are the main requirements for prevention of contamination. The HACCP concept may be useful for decreasing the growth rate of *Salmonella* and other microorganisms by maintaining temperature.

CONCLUSION AND SUGGESTIONS

Chlorine was found effective for decreasing specific growth rate especially for TPC, coliform and *E. coli*. Storage time at -20°C was most effective in decreasing the SGR for TPC, Coliforms, and especially for *E. coli* and *Salmonella*.

Salmonella was still positive after 4 weeks storage at -20°C for samples with or without chlorine treatment.

It can be suggested that prevention of contamination by application of GMP and decreasing growth rate of microorganisms by maintaining low temperatures using the HACCP concept may be useful.

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THE PATTERN OF POST-MORTEM BIOCHEMICAL AND MICROBIOLOGICAL CHANGES IN FARMED TIGER SHRIMP *(Penaeus monodon* Fab.) AT AMBIENT TEMPERATURE

by

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ABSTRACT

An experiment has been carried out to determine the pattern of post-mortem biochemical and microbiological changes of farmed tiger shrimp *Penaeus monodon* Fab.) at ambient temperature (27°C, Rh 75-85%).

Early post-mortem (2-4 hours), bacterial load (total plate count/TPC) increased significantly, and reached a maximum level when putried odour started to develop. Fishy and putrefactive odours arose when TPC reached 10^6 cells/g and 10^7 cells/g, respectively.

The fishy odour started to develop when the content of total volatile base (TVB) was 30.44 mgN%, trimethylamine (TMA) was 12.89 mgN%, TVB/AAN ratio was 0.25 mgN/mM, and pH value was 7.1. When the development of putrefactive odour occurred, TVB, TMA, TVB/AAN ratio, and pH were 31.28 mgN%, 22.31 mgN%, 0.28 and 7.2 respectively. For industrial purposes, TMA content of 22.3 mgN%, TVB content of 30 mgN% and TVB/AAN ratio of 0.28 mgN/mM (as well as the beginning of putrefactive development), are recommended as limits of acceptability of un-iced brackishwater shrimp kept at ambient temperature.

INTRODUCTION

In Indonesia, shrimp are by far the most important export fishery commodity providing about 70 percent of export value in 1988. Black tiger shrimp *(Penaeus monodon)* is the important species contributing at least 38 percent of shrimp export value in 1986. Most of it (75 percent) was produced by brackishwater culture.

In spite of their value for export, relatively little attention has been given to post-harvest handling of brackishwater shrimp in Indonesia. Differences exist in environmental conditions (pond compared to marine) such as salinity, temperature, microbial population, organic and inorganic matter. Information on how these environmental conditions affect storage life is lacking.

Traditionally shrimp are harvested at night and often kept at ambient temperature for 6-8 hours or longer prior to icing. This is not only because of the difficulty in finding ice but also for the purpose of avoiding the water in weighing. Handling malpractices vary from packing shrimp in plastic baskets or drums or sacks, washing shrimp with improper water, to improper icing.

Post-mortem experiments with shrimp have been more concerned with icing than delayed icing (Bailey *et al.,* 1956; Bailey *et al.,* 1960; Bustaman *et al.,* 1985; Chang *et al.,* 1983; Cobb and Vanderzant, 1975; Cobb *et al.,* 1976; Matches, 1980; Matches, 1982; Pedraja, 1970; Tazwir *et al.,* 1986). The important data on the effects of delayed icing or prolonged exposure of brackishwater shrimp to high temperatures, especially in Indonesia, is not available.

An experiment was initiated to find out the pattern of post-mortem changes of farmed black tiger shrimp *(Penaeus monodon* Fab.) at ambient temperature.

MATERIALS AND METHODS

Live farmed tiger shrimp *(Penaeus monodon* Fab.) were obtained from Teluk Naga (Tangerang) and Kamal (Jakarta). Mean weight of the shrimp taken after 14-16 weeks was 44.72 g (flesh 22.32 g) and mean total length was 20.54 cm.

The shrimp were transported live in aerated pond-water to the Research Station for Marine Fisheries Slipi in Jakarta. They were then placed into wood tanks containing seawater with salinity of 27-30 ppt. Immediately after that about 2.5 kg of live shrimp (around 60-70 shrimp) were removed from the tanks and put into plastic baskets (55x40x20 cm^3). Those shrimp were sprinkled with seawater from the tank, drained, and left at ambient temperature for 0.5-1 hour or until they died. The animals were then placed into plastic baskets (45x30x15 cm^3), covered by other plastic baskets to protect from flies, and finally left at ambient temperature (27°C, Rh 75-85%) for 16 hours.

During this period, the shrimp were taken and assessed every two hours for post-mortem changes by sensory, biochemical, and microbiological tests. Biochemical parameters analyzed were total volatile bases (TVB; microdiffusion method), trimethylamine (TMA; microdiffusion method), and free alpha amino nitrogen (AAN; microdiffusion method). Moisture content and pH values were also determined (Anonymous, 1974). Bacterial load was measured using Total Plate Count (TPC) Method (Anonymous, 1974).

For sensory assessment (freshness, including colour, odour, flavour, consistency, and texture which were evaluated by descriptive methods) sampling was every two hours up to 16 hours. Samples for flavour attributes were prepared by dipping the samples in boiling water for about 5 minutes.

RESULTS AND DISCUSSION

1. The Pattern of Shrimp Freshness Changes

The changes of shrimp freshness during post-mortem at ambient temperature are summarized in Table 1. The earliest change recognized was the development of a reddish colour at the shrimp head 4-5 hours after death. While the reddish colour developed, the eyes became dull. After 6-8 hours, the reddish colour spread to the edge of the abdomen and walking legs whereas the dullness of the eyes continued to develop and the flesh became dull. However, the characteristic dark band of the black tiger shrimp was still distinct. As the changes continued, the reddish colour spread widely to all parts of the shrimp including the flesh.

Another change in the early post-mortem period was a fishy odour that developed after 4-5 hours. This odour continued to develop and tended to be slightly putrefactive at the head, especially 6-8 hours after death. In spite of developing a putrefactive odour, 4-5 and 6-8 hours after the death, shrimp flavour was still good and sweet. About 10-12 hours after death the putrefactive odour of the head, as well as the abdomen, was stronger.

According to Cobb and Vanderzant (1975), the freshness of the shrimp was characterized by a species specific odour. Any abnormal odour of shrimp, interpreted as a putrefactive odour, can be used as the reason for rejection, but a fishy odour is still tolerated. It can be tolerated only if a fishy odour could not be identified as the early deteriorative odour (Matches, 1982); Reilly *et al.,* 1987). Physically damaged shrimp should be rejected even though odour and colour are still accepted. Shrimp should also be rejected if there is evidence of reddish colour or black spot even if the odour is still acceptable.

Among the criteria used to evaluate shrimp freshness, odour is the most important, followed by colour. However, the existence of reddish colour at the head, if it does not spread to abdomen, can be manipulated by removing the head to produce headless shrimp. Therefore, it seems that odour could be used as the first major indicator to evaluate shrimp freshness.

Moreover, the earliest changes being recognized, and correlated to deterioration, are odour (slightly putrefactive) and colour (reddish at the upper part of the shrimp head). Those changes could be detected at least 6-8 hours after death. At the same time the texture is slightly loose.

In those conditions, it is suspected that bacterial deterioration occurs and the shrimp are easily spoiled. It means that those shrimp should be rejected for industrial purposes. It is very risky to freeze such material. Furthermore, holding shrimp un-iced at ambient temperature should be no longer than 4 hours. However, as the flavour is still good after 6-8 hours of the death, those shrimp can be tolerated for direct or local consumption. Besides, the occurrence of fishy odour and reddish colour at the upper part of the shrimp head could be used as the first warning prior to rejection.

Table 1 The Changes of Farmed Tiger Shrimp Freshness held at Ambient Temperature

Time (hours) after death	Description
3	No important changes of colour, brightness, and other parameters were recognized
4-5	Reddish colour slightly developed at the upper part of the head. Small white spot appeared. Shrimp brightness became dull and the surface dried. Eye brightness decreased, fishy odour developed especially at the head. Flavour was still sweet, meaty and crabby.
6-8	Reddish colour at the head, spread to the edge of abdomen shell, carapace and walking legs. Blue-yellow pattern as a characteristic of tiger shrimp species was still distinguished. Flesh brightness decreased. Eyes became dull and easy to push. Abdomen elasticity started to decrease. Fishy odour was stronger, and tended to be slightly putrefactive, especially at the head. Fishy odour was also recognized at the abdomen. Flavour was slightly sweet to bland and moist. Flesh was firm and elastic.
12-13	Almost all the shrimp head was red as well as other parts. Eyes dull. Abdomen elasticity was weak. Tissue between head and abdomen was very easy to tear. Spoilage odour was very strong. Off-flavour was easier to recognize. Flesh was very moist and soft.
10-12	Connective tissue was weak, and the tissue between head and abdomen was easy to tear. The carapace can be removed easily. Putrefactive odour was recognized with the head odour strongly putrefactive. Flavour was still acceptable but tended to bland or neutral. Uncertain off-flavour was detected. Flesh was moist and texture was soft.
14-16	Shrimp coloured red, flesh became reddish. Abdomen was very weak and moist. All part of shrimp spoilage. Spoiled or bitter flavour became stronger and flavour evaluation was then stopped. Shrimp flesh was damaged.

2. Bacterial Growth

The bacterial load (TPC) of shrimp used for this experiment was very low as shown in Table 2, i.e., less than 10^2 cell/g. This indicated that shrimp quality was high. The bacteria grew rapidly from the second hour after death and rapidly reached a maximum count in 6 hours (10^7 cell/g of shrimp flesh) when a putrefactive odour was detected. This result was in accordance with previous work by Cobb and Vanderzant (1975) who stated that when a putrefactive odour was detected in iced shrimp, the bacterial count was about 10^6-10^7 cell/g of shrimp flesh.

However, after 10 hours at ambient temperature the bacterial count tended to be stable (Figure 1). It seems that bacterial growth was equivalent to bacterial death. This phenomenon could have been caused by antagonism among bacteria by competitive utilization of nutrients and production of antimicrobial substances as was suspected by Cobb and Vanderzant (1971) for shrimp inoculated with *Coryneform*.

Table 2 Bacterial Load (TPC) on Farmed Black Tiger Shrimp held at ambient temperature[1]

Time (hours)[2]	TPC (x 10^4)[1]	Sensory Assessment
0	< 0,01[3]	- characteristic colour and odour
2	2,37	- characteristic colour and odour
4	113,00	- reddish at the head, fish odour
6	5500,00	- reddish colour wider, odour slightly putrefactive
10	200,00	- reddish colour spread to walking legs, abdomen, putrefactive odour
12	205,00	- red colour spread to all parts, off-odour very strong
16	450,00	- all red, off-odour very strong

3. The Pattern of TMA and TVB Changes

TMA content rapidly rose 2 hours after death to a maximum level of 22 mgN% when a putrefactive odour developed (6 hours) and then tended to slightly decrease when the putrefactive odour was clearly detected (Figure 1). When fishy odour became evident (4 hours), TMA content was 12.89 mgN%. This decrease might be related to the fact that TMA is water soluble (Regenstein *et al.,* 1982). At that time, the shrimp samples were draining and TMA might also drip out with the liquid.

According to Chang *et al.* (1983), if TMA content was more than 5 mgN per 100 g of shrimp flesh, the shrimp are considered to be spoiled. As shown in Table 3, TMA content was 12.89 mgN% after 4 hours at ambient temperature. It means that TMA level was much higher than the limit of acceptability as recommended by Montgomery *et al.* (1970); in Chang *et al.*, (1983).

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[1] Counted since death

[2] Average of two replications (cell/g of shrimp flesh)

[3] Not detected at 10^{-2} sample solution

Table 3. Biochemical Changes of Farmed Black Tiger Shrimp held at Ambient Temperature

Indicators	Time (hours)							
	0	2	4	6	8	10	12	16
Moist.cont.(%)	77.69	77.63	75.87	77.38	75.11	78.45	75.55	75.23
pH	6.43	7.10	7.54	7.20	7.26	7.19	7.21	7.24
TMA (mgN%)	2.36	1.12	12.89	22.31	21.56	-	21.14	21.56
TVB (mgN%)	15.97	22.92	30.44	31.28	52.63	42.45	96.19	124.72
AAN (aM%)	127.56	112.53	123.9	113.22	120.4	109.1	119.7	123.90
TVB/AAN (agN/aM)	0.13	0.20	0.25	0.28	0.44	0.39	0.80	1.01

Chang *et al.*, (1983) suggested that TMA was not a good indicator for shrimp spoilage, but these authors used different analytical methods and shrimp species, as well as temperature of storage. However, for the tropical brackishwater black tiger shrimp, which were kept un-iced at ambient temperature, as shown in Figure 2, and taking the development of fishy or putrefactive odour into account, TMA change seems to be a potential spoilage indicator, at least until a slightly spoiled odour was detected at the head (6 hours). If these criteria are accepted, the maximum TMA for the borderline quality could be 22 mgN%.

Slightly different from TMA content which increased rapidly after 2 hours of the shrimp death, TVB content increased rapidly in two steps. The first rapid increase of TVB content occurred after 4 hours when the concentration reached 30.44 mgN% was coincidental with the detection of the first fishy odour. The second rapid increase took place after 10 hours when the putrefactive odour was clearly detected and the concentration reached 42.45 mgN% (Figure 3). TVB content still tended to increase after 16 hours.

Like TMA, TVB is often used as an indicator for shrimp spoilage. Montgomery *et al.*, (1970), as quoted by Cobb and Vanderzant (1975), reported that Australia and Japan have been using TVB and TMA content of 30 mgN% and 5 mgN% respectively as the limits of acceptability of peeled shrimp as reported by Gagnon and Feller. Fieger and Friloux (1954), and Iyengar *et al.* (1960) all cited by Cobb and Vanderzant (1975), suggested that TVB and TMA content of shrimp were not good indicators of iced shrimp quality because of leaching due to the washing effect of melting ice.

However, Figure 3 suggests that TVB and TMA contents might be used as indicators for spoilage for brackishwater black tiger shrimp kept un-iced at ambient temperature, especially if the odour changes were taken into consideration.

If a TVB content of 30 mgN% can be recognized as the limit of acceptability, un-iced shrimp kept at ambient temperature should be rejected after 4 hours. This agrees with the limit of acceptability using TMA content. In spite of the rejection by TMA or TVB indicator, shrimp were still acceptable due to the fact that the putrefactive odour was not detected until kept at ambient temperature for more than 6 hours. At that time TVB content was 30.44 mgN% and a slight fishy odour was detected. It is likely that TMA and TVB is related to the fishy odour rather than putrefactive odour.

As a result, with the assumption of TVB content of 30 mgN% as a limit of acceptability, un-iced black tiger shrimp could be rejected for industrial purposes, i.e., when fishy odour was detected. This fishy odour indicates an early stage of spoilage; in a further 2 hours the odour was putrefactive (TVB 31.28 mgN%, TMA 22.31 mgN%). So that fishy odour might be used as an early warning.

4. pH Value Changes

Another spoilage indicator of shrimp is pH value (Iyengar *et al.*, 1960; *in* Cobb and Vanderzant, 1975), and usually pH value of 7.6 is considered to be borderline for quality of fishery products.

Up to 4 hours at ambient temperature, pH value rapidly rose to a maximum value of 7.54. But after that time pH value decreased to 7.2 and then tended to be relatively stable (Figure 4). The decrease of pH value could have been caused by producing lactic acid from glycogen degradation.

When fishy and putrefactive odours became evident, pH values were 7.54 and 7.2 respectively. It means that pH value of 7.54 would express the early spoilage stage of farmed black tiger shrimp kept at ambient temperature. However, shrimp could be categorized as spoiled when pH value was 7.2. Taking both pH values and sensory assessment into account, 7.2 may be a safe pH value to determine whether the shrimp was spoiled. Moreover, pH values should be combined with other indicators such as TVB, TMA, and especially sensory assessment.

5. TVB/AAN Ratio

Changes of TVB/AAN ratio were similar to the pattern of TVB changes, i.e., increased rapidly after 10 hours at ambient temperature (0.39) when the putrefactive odour was clearly detected (Figure 5). When the fishy odour became evident (4 hours) and the putrefaction was first detected (6 hours), TVB/AAN ratio were respectively 0.25 and 0.28 mgN/mM. This ratio tended to increase rapidly after 16 hours.

According to Cobb and Vanderzant (1975), TVB/.AAN ratio may be a more accurate indicator of shrimp quality than bacterial count. These authors also stated that TVB/AAN ratio may be valuable in assessing shrimp quality and that when the shrimp odour was slightly putrefactive, the TVB/AAN ratio was 1.5 mgN/mM.

As shown in Table 3, the first fishy odour was detected with a TVB/AAN ratio - of 0.25 mgN/mM, and when slight putrefaction was detected (6-8 hours) ? the ratio was around 0.28-0.44 mgN/mM. These results were much lower than that of Cobb and Vanderzant (1975). Until 16 hours after death the ratio (1.01 mgN/mM) was still less than 1.5 mgN/mM.

This phenomenon is likely because the initial AAN content was high (127.56 mM%). Cobb and Vanderzant (1975) also reported that the initial AAN content of refrigerated seawater shrimp was more than 60 mM% while TVB content of spoiled shrimp was only 30 mM%. With a TVB content which was more than 30 mgN%, TVB/AAN ratio would be high. However, Vanderzant *et al.* (1973) stated that initial AAN contents of farmed brackishwater shrimp were high; consequently, TVB/AAN ratio was low, i.e., around 0.90 mgN/mM. In this case, Vanderzant *et al.* (1973) reported spoilage patterns differing greatly from those usually observed for wild shrimp. For such specialized cases, the spoilage value of the TVB/AAN ratio may have to be adjusted.

Besides, differences in environmental conditions may cause differences in AAN level. For example, higher salinities may promote higher AAN levels (Cobb and Vanderzant, 1975; McCoid *et al.*, 1984). Differences in water temperature may cause differences in ammonia-producing enzymic levels. Therefore, TVB content may be different. Besides, the existence of natural bacteria on shrimp was also affected by temperature and salinity (Cobb and Vanderzant, 1975).

Tropical farmed black tiger shrimp with a TVB/AAN ratio around 0.28-0.44 mgN/mM (0.36 mgN/mM on average) started to be slightly putrefactive, and when TVB/AAN ratio was 0.25 mgN/mM a fishy odour was detected. It seems that if TVB/AAN ratio of un-iced farmed black tiger shrimp was 0.36 mgN/mM or more, the shrimp was unfit for export.

SUMMARY

1. The freshness of un-iced shrimp held at ambient temperature (27°C, Rh 75-85%) changes rapidly. The first changes recognized are fishy odour and reddish colour on the upper part of the head. Fishy odour gradually became putrefactive, while the reddish colour spread to other parts of shrimp body.

2. There are two dominant sensory attributes for evaluation of shrimp quality, namely colour and odour. For industrial purposes, un-iced farmed black tiger shrimp kept at ambient temperature should be rejected if a slightly putrefactive odour or reddish colour is evident. If a fishy odour is detected, it is necessary to take into account other indicators, such as biochemical and microbiological indicators, for making a decision.

3. When fishy odour and reddish colour of the head became evident, TCP increased up to 10^6 cell/g of shrimp flesh. At that time, TVB and TMA content were 30.44 mgN% and 12.89 mgN% respectively, while TVB/AAN ratio and pH value were respectively 0.25 mgN/mM and 7.1. TVB and TMA are related to the fishy odour rather than the putrefactive odour. Initial AAN content of farmed black tiger shrimp from tropical water was high (127.56 mM%).

4. When the putrefactive odour was detected for the first time, TPC sharply increased to a maximum level (10^7 cell/g) after 6 hours the shrimp kept at ambient temperature, whereas TVB and TMA content were 31.28 mgN% and 22.31 mgN% respectively, TVB/AAN ratio and pH value were 0.28 mgN/mM and 7.2. It seems that both TVB and TMA level (30 mgN% and 22.3 mgN%) might be used as a limit of acceptability of shrimp for industrial purposes.

5. As a whole, it is a high risk to leave shrimp at ambient temperature un-iced for more than 4 hours. They should be iced immediately.

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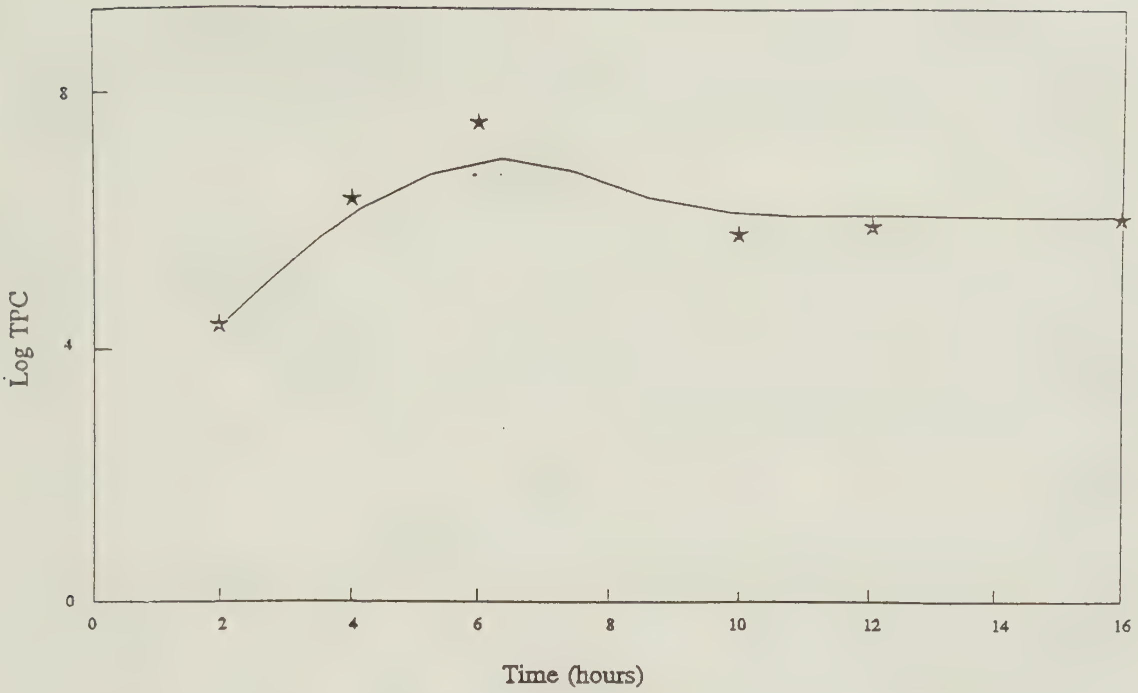

Figure 1. The pattern of logarithmic change of bacterial load on Farmed Black Tiger Shrimp held at ambient temperature

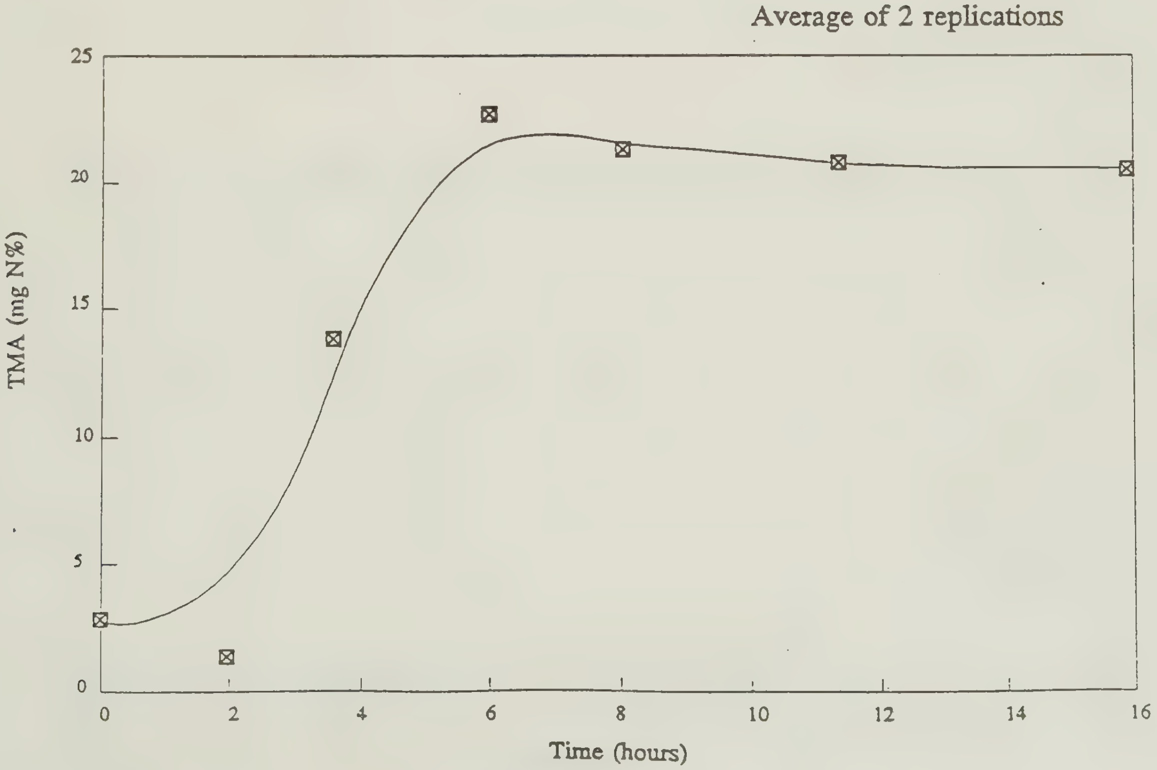

Figure 2. The pattern of TMA changes on Farmed Black Tiger Shrimp held at ambient temperature

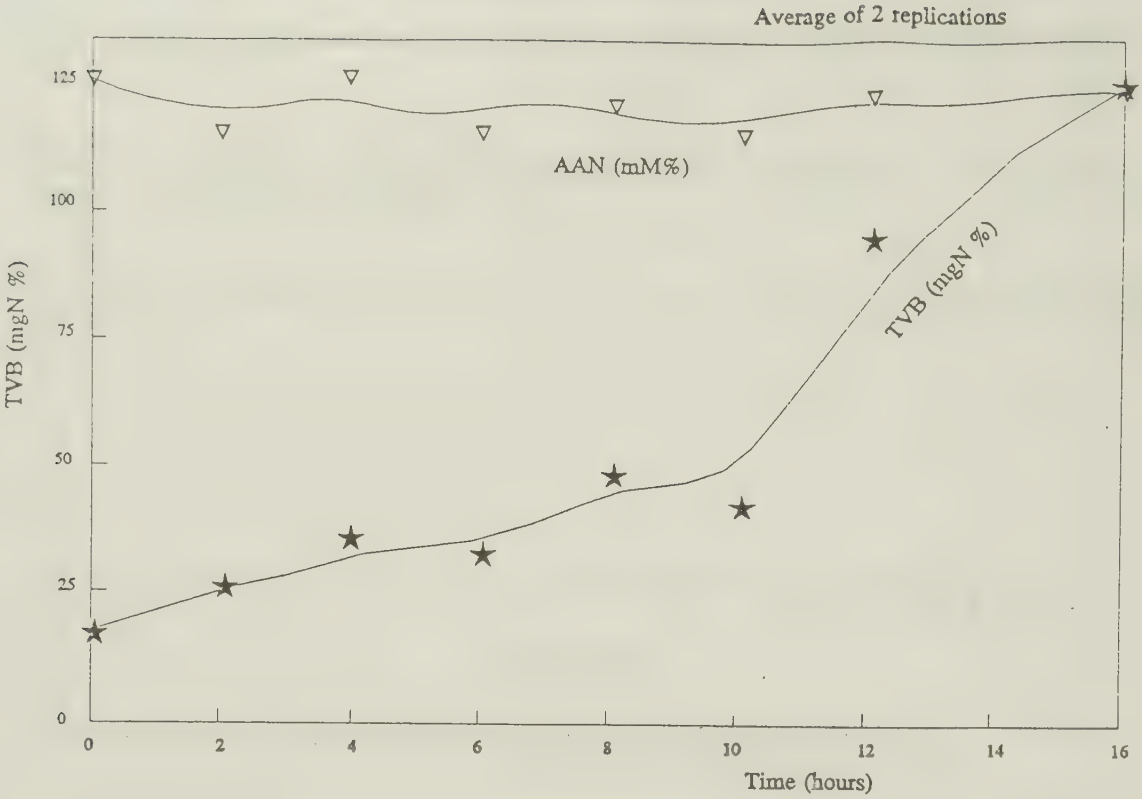

Figure 3. The pattern of the TVB and AAN ratio changes on Farmed Tiger Shrimp held at ambient temperature

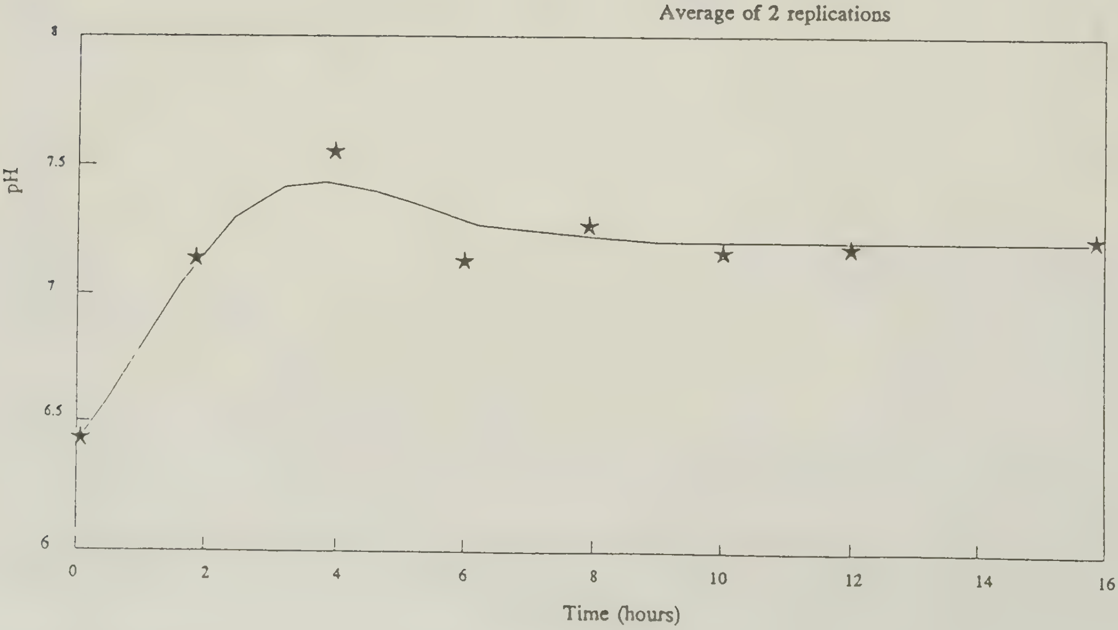

Figure 4. The pattern of pH changes of Farmed Black Tiger Shrimp held at ambient temperature

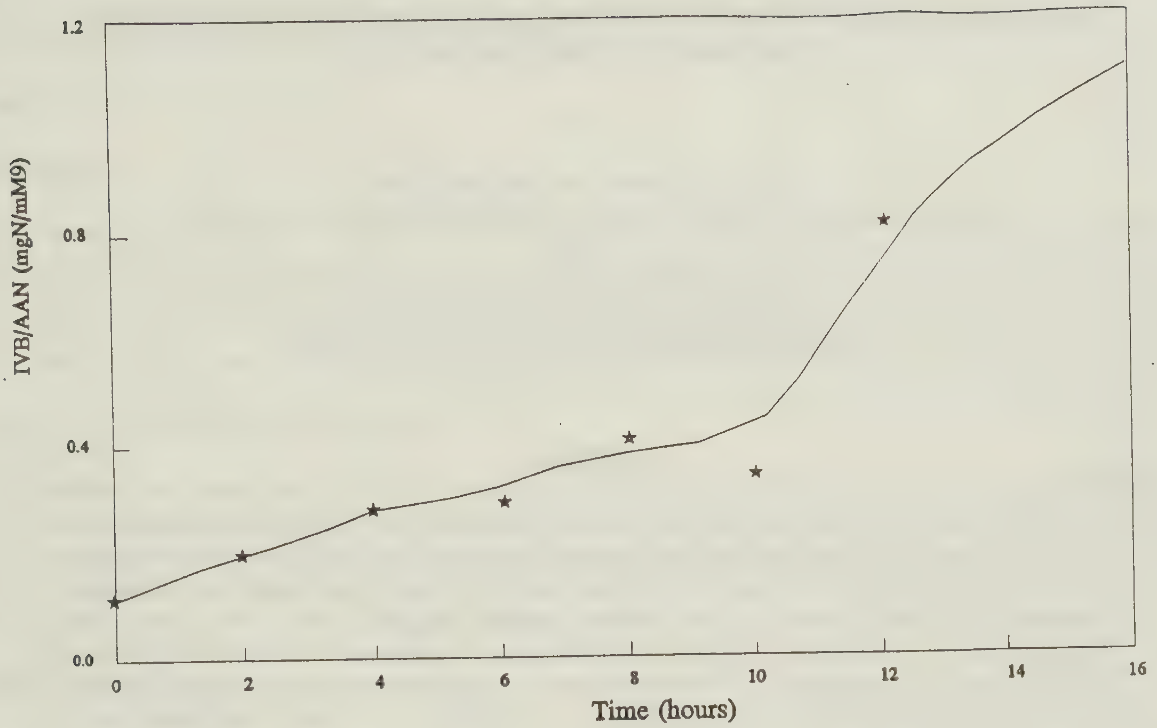

Figure 5. TVB/AAN ratio changes of Farmed Black Tiger Shrimp held at ambient temperature

BACTERIAL LOAD OF NORMAL, MOULTING AND DELAYED MOULTING FARMED TIGER SHRIMP (*Penaeus monodon* Fab.)

by

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ABSTRACT

An experiment has been conducted to find out bacterial distribution on farmed tiger shrimp (*Penaeus monodon* Fab.) as well as bacterial load in normal and moulting shrimp and shrimp where moulting is delayed. The results showed that, in normal samples, the part containing the lowest aerobic bacterial load (TPC) was flesh, while both the head and the legs were highest. Anaerobic bacteria (APC) were mostly found in the head. *Coliform* bacteria in the head and legs were relatively high, but they were absent in the flesh. In addition, *E. coli* and *Salmonella* were absent in all samples.

The TPC in shrimp where moulting was delayed was higher than that in moulting and normal shrimp. Bacterial load in moulting shrimp (1.3×10^6 cells/g of flesh) was much higher than that in flesh of normal shrimp (under 10^2 cell/g). Besides, APC in the shrimp flesh all samples was very low, i.e. under 10^2 cell/g. This suggests that the shrimp shell might protect the flesh from bacterial contamination.

INTRODUCTION

The most important species of Indonesian shrimp exports is black tiger shrimp (*Penaeus monodon* Fab.). Culture contributed 74.6 percent of total product of black tiger shrimp in 1986.

Shrimp quality is an important supporting factor for export and is affected by the conditions of pond culture. During culture, the most critical period is the time of moulting which takes place periodically.

The physiological process takes place by softening the inner layer of the old shell, and the growth of the new one. It is the presence of moulting of a thin and elastic new shell which allows for expansion of the body as growth in size of shrimp takes place (Dennell, 1960; Passano, 1960; Poernomo, 1979; Pasaribu, 1981; Ruello, 1981; Ruello, 1976; Smith and Dall, 1985; Wassenberg and Hill 1984). However, this natural process sometimes fails resulting in a shrimp with delayed moulting.

The soft, thin shell probably makes the shrimp more prone to bacterial contamination during harvesting and handling. Shrimp that are moulting fetch a lower price and may be rejected by the processing industry because their quality decreases rapidly.

The price for shrimp where moulting is delayed is also much lower than for normal shrimp and they are often rejected because of suspicion of higher bacterial load. In traditionally managed pond shrimp culture some 5-10% of the shrimp show delayed moulting.

Previous investigations have concentrated on reducing bacterial load to lengthen shelf life (Peranginangin *et al.*, 1973 and 1985; Sarnianto *et al.* 1985). These investigations were conducted to determine the bacterial loads of normal, moulting and delayed moulting shrimp in order to see if the results would be useful for quality control of shrimp in Indonesia.

MATERIALS AND METHODS

Materials

Live farmed tiger shrimp were obtained from semi-intensive ponds in Teluk Naga (Tangerang) and Kamal (Jakarta). Mean weight of the shrimp at age 14-16 weeks was 44.72 g (flesh 22.32 g) and mean length 20.54 cm). Normal shrimp were mature shrimp ready for harvest with hard shell, well filled with flesh. Moulting shrimp were mature, based on age and size, but with thin and soft shell during the moulting period. Delayed moulting shrimp were similarly mature based on age and size but had failed to moult for some time.

Transporting and holding live shrimp

The live shrimp were transported to the laboratory within 1.5 hours in a large box containing aerated brackish water with ice added to maintain the temperature in the range 25-17°C. In the laboratory shrimp were kept in holding tanks ($1x1x0.5m^3$) containing clean aerated sea water. They were fed twice a day with commercial shrimp feed (about 5% of body weight). The seawater was cleaned every two days and about one third replaced.

Moulting and delayed moult samples

Because of the difficulty of finding these samples and the risk of damaging them during transport they were obtained by manipulation of the conditions in the laboratory.

Moulting shrimp were produced by gradually increasing the salinity to 32-35 ppt. Feeding was stopped for two days and subsequent daily checking at the bottom of the tanks identified shrimp with soft moulting shell. They were removed to a separate tank and analyzed the following day.

For the delayed moult the salinity was increased to 32-35ppt and feeding reduced gradually to once per day. Cleaning of the water was reduced to every few days. These treatments were introduced gradually to reduce stress. The moulting shrimp were removed and after 2-3 months the remainder were considered delayed moulting.

Sample preparation

The cephalothorax, abdomen, flesh and swimming and walking legs were examined for aerobic and anaerobic counts by pour plating. Normal shrimp were also examined for coliforms, *E. coli* and *Salmonella*. About 10 g of shrimp (25 g for *Salmonella*) were taken aseptically with 90 ml physiological solution and blended in a stomacher bag for 1 minute. For the leg samples about 2 g of legs were removed aseptically and shaken in a bottle with 18 ml of sterile saline.

Microbiological procedures

Bacto nutrient Agar (Difco) was used for the aerobic plate counts with triplicate pre-poured plates (plus 0.1 ml of diluted sample) incubated at 37°C for 48 hours. For the anaerobic plate counts plates were incubated for the same time temperature but in anaerobic jars with a gas pack and indicator (gas generating kit, Oxoid Ltd.).

For *E. coli* and coliforms the samples were pre-enriched in McConbey Broth (1:1000 dilution) for 48 hours at 37°C and then analyzed in multiple tubes by MPM (Anon, 1974). For *Salmonella* Bacto Selinite Cystine Broth (SCB), Bacto Desoxychlorate Citrate Agar (DCA) Bacto Xylase Desoxycholate Agar (XLD) and TSIA were used. Ten ml of suspended sample was homonogenized in SCB and incubated at 37°C for 24 hours. A loopful of the suspension was streaked on DCA and XLD and incubated again at 37°C for 24 hours. Putative salmonella colonies (brown points in transparent DCA black in XLD) were transferred to TSIA for a further 24 hours at 37°C. Suspect colonies were confirmed by biochemical tests.

RESULTS AND DISCUSSION

The results are presented in Table 1.

Table 1 Microbiological load of moulting and delayed moulting shrimp (cell/g part of shrimp body)

	TPC	APC	Coliform	E. Coli	Salmonella
Normal shrimp					
Whole	1.58 x 10^5	6.49 x 10^4	6.8 X 10^3	negative	negative
Head	2.92 x 10^4	4.57 x 10^3	1.7 x 10^4	negative	negative
Tail	5.08 x 10^3	$<10^2$	4.0 x 10^4	negative	negative
Flesh	$<10^2$	0	negative	negative	negative
Legs*	2.21 x 10^4	$<10^2$	1.2 x 10^3	negative	negative
Moulting shrimp					
Whole	2.33 x 10^5	$<10^2$	n.e.	n.e.	n.e.
Head	3.71 x 10^5	$<10^2$	n.e.	n.e.	n.e.
Tail	9.90 x 10^4	$<10^2$	n.e.	n.e.	n.e.
Flesh	1.30 x 10^5	$<10^2$	n.e.	n.e.	n.e.
Legs*	3.88 x 10^4	0	n.e.	n.e.	n.e.
Delayed moulting shrimp					
Whole	4.15 x 10^4	$<10^2$	n.e.	n.e.	n.e.
Head	2.26 x 10^5	$<10^2$	n.e.	n.e.	n.e.
Tail	3.75 x 10^4	0	n.e.	n.e.	n.e.
Flesh	5.55 x 10^3	0	n.e.	n.e.	n.e.
Legs*	2.92 x 10^5	0	n.e.	n.e.	n.e.

n.e. = not examined
* = cell/shrimp

Aerobic counts (TPC) The TPC of normal whole shrimp 1.58 x 10^5 colonies/g compares unfavourably with the Indonesian Export Standard of 10^5 cells/g. Levels in the cephalothorax, tail and flesh were somewhat lower. For the legs the figure of 2.2 x 10^4 is number of colonies for shrimp. However, Peranginangin (1982) reported that tropical shrimp generally had counts of 5 x 10^2 to 1.7 x 10^5 colonies/g of flesh while levels in cold water shrimp were of the order of 10^6 colonies/g. On this basis the normal shrimp in this study could be considered to be of high quality.

It appears that the head and legs were the parts most easily contaminated either in the pond or by harvesting and handling. This is presumably as a result of their anatomical structure. Shrimp gills on either side of the cephalothorax consist of 17 pairs of feather-like processes. Water is pumped over the gills in a posterior-anterior direction (Mahardono *et al.* 1981; McLaughlin 1983). all particles and microorganisms in the water can therefore be filtered out by the gills making it likely that the head will have a higher count. The digestive system in the head also contributes to the bacterial load from feed etc.

The anatomical structure of the small walking legs and the swimming legs also tends to accumulate bacteria. The walking legs are wide enough to provide a large area of contact while the swimming legs are hairy giving possibilities of entrapment. The low TPC of the shrimp flesh is an indication of environmental contamination.

In both the moulting and delayed moulting samples the TPC's were substantially higher with a higher level in the flesh of moulting shrimp indicating more rapid invasion across this shell.

Anaerobic counts (APC) on normal shrimp were higher for the whole shrimp and cephalothorax which may be indicative of surface contamination of the gills etc. The anaerobic levels for the moulting and delayed moulting shrimp were lower

E. Coli and *Salmonella* were not found in any sample, which supports previous studies by Peranginangin (1985) and Sarnianto *et al.* (1985). However, coliform counts of up to 6.8×10^3 are indicative of significant environmental pollution.

There are more opportunities for contamination of cultured shrimp, during the growing phase, than for marine capture shrimp due to the proximity of culture areas to human settlements. The impacts have been considered by Hobbs and Hodgkiss (1982), Kawabata (1962), Guelin (1962), Buttianx (1962) and Shewan (1962). The presence of bacteria may result from their natural existence in the gut such as evidence of micrococcus presented by Shewan (1962). The natural flora of the gut would presumably be less dangerous than contaminating species. Ruello (1981) has suggested that bacteria in tropical shrimp will die quickly if they are chilled or frozen.

In summary, the bacterial condition of live normal shrimp could be described as high quality while counts from moulting and delayed moulting shrimp were higher: indicative of transfer across the thin shell and presence in the cavity of the delayed moulters.

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MICROBIAL FLORA OF KARACHI COASTAL WATER SHRIMP (*Penaeus Merguiensis*) AND ROLE IN SHRIMP QUALITY DETERIORATION

by

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ABSTRACT

Bacterial counts were made over a period of two years for an important shrimp species *(Penaeus merguiensis)* in Karachi coastal waters. Bacteria were isolated and identified. Total number of bacteria were found to be remarkably equal at 30°C and 25°C. The count at 30°C ranged from 3.2 x 10^5 to 4.7 x 10^7CFU/g (Median 2.6 x 10^6 CFU/g). The bacterial flora in order of predominance was *Vibrio, Micrococcus, Pseudomonas, Moraxella, Flavobacterium, Bacillus, Alteromonas* and *Acinetobacter*. The corresponding count at 25°C ranged from 5.3 x 10^5 to 8.5 x 10^7 CFU/g (Median 3.1 x 10^6 CFU/g), the flora was composed of *Vibrio, Moraxella, Micrococcus, Pseudomonas, Flavobacterium, Bacillus, Alteromonas* and *Acinetobacter*, in order of predominance.

Changes occurring in bacterial populations and in generic composition of bacterial flora during storage in ice (0°C), 10°C and 22°-30°C (room temperature) were also studied. The bacterial population increased from an initial count ranging from 5.5 x 10^5 to 4.7 x 10^7 CFU/g to 109 CFU/g after 16 days of storage in ice 0°), 10^9 CFU/g after 12 days at 10°C and to 10^{10} CFU/g, after 24 hours, at room temperature. At the end of the storage period the dominant organisms in shrimp during ice storage (0°) were *Alteromonas, Moraxella, Pseudomonas* Group II and III and *Micrococcus*. *Micrococcus* dominated at 10°C together with the flora found at 0°. At room temperature (22°-30°C), the mesophyllic flora, composed of *Vibrio, Pseudomonas* Group III, *Bacillus* and *Micrococcus* was dominant. Boiled and sterile raw shrimp muscles were inoculated with pure cultures of *Alteromonas, Pseudomonas* Group II and III, *Moraxella* and *Micrococcus* isolated from the shrimp (*Penaeus merguiensis*) during previous studies to determine the spoilage potential of these species following 14-day storage at 0°C. *Alteromonas putrefaciens* strains produced putrid odours; slimy textures, increases in pH, trimethylamine, and total volatile bases. No changes were found in inoculated controls. *Pseudomonas* Group II also proved to have spoilage potential. *Pseudomonas* Group III, *Moraxella* and *Micrococcus* produced changes, but to a lesser extent that *Alteromonas* and *Pseudomonas* Group II.

INTRODUCTION

The coast of Pakistan is a rich source of high quality seafood. Shrimp, being a highly perishable product and also the main item of export from the sector, demands careful handling from harvesting to processing. As with many other foods, quality specifications, particularly bacteriological guidelines, are being introduced especially where international trade is concerned. In order to arrive at realistic and practical standards, it is essential that there is thorough understanding of the numbers and types of bacteria present on raw material.

It is now well recognised that marine catches after harvest must be properly stored in ice, as early as possible, to prevent rapid bacterial growth which is one of the major causes for the deterioration of quality during subsequent storage. In commercial situations, however, this is not practicable.

Trawling in Pakistan tropical waters normally takes about 10 hours. A shrimp trawl contains shrimp, small fish and other marine animals. Before icing, shrimp are separated from the trash fish and are usually deheaded. Thus, icing which is of vital importance is undertaken usually several hours after catch. During this period the shrimp may be held at ambient temperatures ranging from 15°-40°C. Furthermore, during handling ashore and in the processing factories, improper ice storage may also be a source of bacterial multiplication. In any event, where shrimp are allowed to remain at warm ambient temperatures or poorly iced, degradative changes begin to take place rapidly. The rate of these changes, as may be expected, is temperature dependent.

It has long been established that rapid fish spoilage, even at cold temperatures, is primarily due to bacterial action. The roles played by different groups of organisms found in Karachi coastal water shrimp are still uncertain. It is generally agreed that at 0°C fish and shrimp become unacceptable organoleptically, biochemically, and microbiologically within two weeks (Shewan, 1962; Shaw and Shewan, 1968; Herbert *et al,* 1979; and Cobb *et al*, 1976.

This paper presents the results of three investigations on Karachi coastal water shrimp (*Penaeus merguiensis*), the marine shrimp of commercial importance in Pakistan: (a) Quantitative and Qualitative Aspects of Bacterial Flora of Karachi Coastal Water Shrimp (Zuberi *et al,* 1985), (b) Effect of Elevated Temperature of Storage on the Bacteriological Quality of Shrimp (Zuberi *et al*, 1989) and (c) Biochemical Changes in Shrimp Inoculated with *Alteromonas, Pseudomonas, Moraxella* and *Micrococcus* species (Zuberi *et al,* 1988).

MATERIAL AND METHODS

Shrimp Samples

Shrimp of commercial important *Penaeus merguiensis* locally known as "Jaira" were selected for study. Samples were obtained from Ibrahim Hydri. These shrimp were brought in by fishermen who operate small boats and generally make short trips, going fishing in the morning and coming back at 4-5 p.m. in the afternoon. The whole trip does not take more than 10 hours. The shrimp were packed in ice immediately after purchase, and examined within one hour of sampling.

A 50 g portion of each deheaded sample with 450 ml of sterile diluent 0.1% peptone (w/v) (Difco), 0.9% NaCl (W/V) was blended for 2 minutes in an "Atomix" blender. Appropriate dilutions prepared in the same diluent were spread plated in duplicate on tryptic soy agar (Difco). Plates were incubated for 48 hours at 30°C and 48 to 72 hours at 25°C.

To determine microbial types 30-40 colonies were picked up at random from countable plates. Gram-positive cocci were identified using the method of Baird Parker (1965). Gram-negative rods were classified by the identification scheme of Shewan *et al* (1960) and Buchanan and Gibbson (1974). Gram+ rods were identified according to Buchanan and Gibbson (1974). *Alteromonas putrefaciens* was identified by H_2S production on peptone iron agar (Difco) (Levin, 1968) and distinguished by the TMAO reduction and ornithine decarboxylase reaction.

Storage changes

Seven experiments were performed at each storage temperature. The shrimp were divided into three groups: one held in ice (0°C), the other two being held at 10°C and at room temperature (22°-30°C).

For ice storage the deheaded shrimp were distributed in perforated polythene bags containing shrimp tails weighing 17-100g. One set of five bags was covered with sterile crushed ice and stored in the refrigerator for 16 days. The shrimp were constantly checked for ice during storage and re-iced when required. The other set of five bags was stored at 10°C in a cold incubator. For room temperature storage, the shrimp samples were kept in covered sterile petri dishes. The samples stored in ice (O°C) and at 10°C were examined bacteriologically after 2, 4, 8, 12 and 16 days of storage. Those stored at room temperature were analyzed after 6 and 24 hours.

Biochemical changes

Sample preparation

The following two methods were used for obtaining sterile shrimp: (1) Shrimp were headed, peeled, and deveined, aseptically, then rinsed with distilled water, 70% ethanol, and sterile deionized water, (2) Shrimp were boiled in 1% NaCl solution, cooled, and peeled, aseptically. These sterilized shrimp were analyzed for total bacterial prior to the start of the experiment. No growth was found in the boiled samples, but growth did occur in three of the washed samples (average count 1.8 x 10^3 colony forming units (CFU/g).

Culture organisms

The following seven cultures were used as test organisms; *Alteromonas,* rough and smooth variants (R and S): *Pseudomonas* Group III; *Moraxella* and *Micrococcus*, yellow and orange varieties. These bacteria were selected as representative groups commonly encountered in spoiled shrimp stored in ice (Zuberi *et al,* 1968). Six strains of each bacteria were isolated from shrimp that had spoiled during storage in ice in previous studies.

Culture organisms preparation

For each experiment, a single bacterial strain was subcultured on nutrient agar slants with 1% NaCl. The slants were incubated for 24 hours at 25°C. The organisms were washed with 10 ml sterile saline, centrifuged at 5,000 for 10 minutes, and washed by resuspension in saline,. Dilutions were made in sterile diluent to obtain concentrations of 10^5 CFU/g, the equivalent found in freshly caught Jaira shrimp (Zuberi *et al,* 1986). The shrimp samples where then contaminated by dipping them in suspensions of pure cultures. The samples were stored in petri dishes at 0°C for 14 days.

Biochemical analysis

Trimethylamine was estimated by Dyer's picric acid procedure (1945), as modified by Murray and Gibson (1972). Total volatile bases were determined according to Cobb *et al,* (1973).

For measurement of pH, 10g of shrimp muscle were homogenized in 20 ml distilled water. The pH was measured using a Cambridge pH meter.

RESULTS AND DISCUSSION

Quantitative and Qualitative Aspects of Bacterial Flora

Table 1 lists the generic composition of bacterial flora of eleven samples of Jaira shrimp. The bacterial cultures were isolated at 30°C. The count ranged from 3.2 x 10^5 to 4.6 x 10^7 CFU/g (Median 2.6 x 10^6 CFU/g). The flora in order of predominance was *Vibrio, Micrococcus, Pseudomonas, Moraxella, Flavobacterium, Bacillus, Alteromonas putrefaciens* and *Acinetobacter.*

Generic composition of bacterial flora of another 12 samples of Jaira is summarized in Table 2. The bacterial cultures were isolated at 25°C. The count ranged from 5.2x10^5 to 8.5x10^7 CFU/g (Median 3.1x10^6 CFU/g). The flora in order of predominance was *Vibrio, Moraxella, Micrococcus, Pseudomonas, Flavobacterium, Bacillus, Alteromonas putrefaciens* and *Acinetobacter.* No qualitative seasonal variation was revealed at either 30°C or 25°C.

About 1500 cultures were isolated at 30°C and 25°C from different samples of shrimp. There was no significant difference in percentage composition and the order of predominance, isolated at 25°C and 30°C, except that the percentage of *Moraxella* was 20 at 25°C as compared to 12.8 at 30°C.

Mesophyllic *Vibrio* dominated in % composition over other organisms both at 25°C and 30°C. During April, May and June, *Vibrio* were isolated in excessive numbers as they were very fast growers at higher environmental temperatures. In a previous study, Qadri and Zuberi (1977) obtained a mean count of 3.8x10^6 CFU/g and 3.5x10^5 CFU/g respectively, on shrimp and fish from Karachi coastal waters.

Out of 108 samples examined 107 (99%) were positive for *Vibrio alginolyticus* and 64 (60%) positive for *Vibrio parahaemolyticus*.

Micrococci were found to be the second dominating organism isolated at 30°C and third at 25°C. They were observed throughout the study with slight variation in percentages and constitute a permanent part 15.7% and 18.2% of the total flora at 30°C and 25°C, respectively.

The genus *Pseudomonas* was the third dominating organism at 30°C and fourth at 25°C (Tables 1 and 2). Most of the *Pseudomonas* isolates were found to belong to Group III according to the identification scheme of Shewan *et al,* (1960). A seasonal effect is noticeable. The organisms appeared in December and January, the coldest months of Karachi winter (10°-20°C), inhibiting the growth of mesophyllic *Vibrio* in most of the samples examined. It has been reported (Shaw and Shewan, 1968) that psychrophilic bacteria are predominant in the genera *Pseudomonas, Moraxella* and *Flavobacterium.* All these organisms were isolated both at 30°C and 25°C. *Flavobacterium* and *Bacillus* genera were also present both at 30°C and 25°C, in low proportion.

Alteromonas putrefaciens was isolated both at 30°C and 25°C again in low proportions. This organism, formerly classified as *Pseudomonas putrefaciens* has been the subject of many spoilage studies (Castell *et al,* 1949; Chai *et al,* 1968; Levin, 1968). It has been reported (Chai *et al,* 1968) that *P. putrefaciens* constitutes one of the major species of spoilage bacteria on haddock fillets. It was observed that the initial population of this organism on fillets of high bacterial quality was uniformly below 4% and most frequently not greater than 1%. During refrigerated storage the organisms increase at a more rapid rate than the total psychrophilic population and constitute 50-90% of the total population when the count exceeds 10^6 CFU/g of the tissue. In the light of the above facts, the presence of these organisms indicates the spoilage potential of the shrimp during ice storage.

The number of types of bacteria on fish at the time of landing depends on many factors such as species, season, fishing grounds, methods of catching, handling on board and the time and temperature of storage (Vanderzant *et al,* 1970). Data are available on bacteria present on freshly caught shrimp from various parts of the world (Green, 1949; William *et al,* 1952; Fieger *et al,* 1958; Carroll *et al,* 1968; Cobb *et al,* 1973 and 1976; Matches, 1982). Total viable count has been reported between 10^2-10^7 CFU/g for freshly landed shrimp. The main group of bacteria on Gulf of Mexico shrimp have been reported to be coryneforms, *Pseudomonas, Moraxella* and *Micrococcus* (Vanderzant *et al,* 1970). Cobb *et al,* (1976) reported *Vibrio, Pseudomonas, Moraxella* and *Acinetobacter* to be the dominant species in fresh Gulf shrimp.

Very little information is available on the microbial flora of tropical shrimp from this region. Furthermore, the type of media and the temperature of incubation used for the isolation of organisms by different workers makes it difficult to compare the results. However, for the sake of discussion, the following comparison may be made.

Shaikhmehmood and Magar (1956) conducted a bacteriological study of Bombay Prawn (*Parapenaeopsis stylifera*). Their 37°C count of unwashed prawn, immediately after landing, ranged from 6.7×10^5 to 7.05×10^5 CFU/g. They also noted the variations in samples collected from catches 5 and 15 hours after landing. Only 40 cultures were isolated, from different parts of the prawn. Organisms belonging to the genera *Achromobacter, Micrococcus, Bacillus, Flavobacterium, Sarcina, Pseudomonas,* and *Aerobacter* were found to be commonly associated with prawns. The reported flora may not be representative of prawn (*P. stylifera*) because only 40 isolates were identified. Vanderzant and Nickleson (1979) reported the microbial count of Gulf of Mexico shrimp at 28°C ranging from $8l7 \times 10^2$ to 1.3×10^6 CFU/g, somewhat similar to the count of Karachi coastal water shrimp.

Results of our studies have revealed the following a) The flora of Karachi coastal water shrimp Jaira (*Penaeus merguiensis* is composed of *Vibrio, Micrococcus, Pseudomonas, Moraxella, Flavobacterium, Bacillus* and *Alteromonas*; b) the bulk of the flora appears to be composed of mesophyllic organisms which grow well at 25°C and 30°C; c) shrimp of this area are dominated by the large numbers of mesophyllic Gram-negative species of *Vibrio* and psychrotrophic species of *Pseudomonas, Moraxella, Acinetobacter, Alteromonas* and *Flavobacterium* in contrast to fish from similar warmer waters where mesophyllic Gram-positive species of *Micrococcus, Bacillus* and *Coryneforms* seem to predominate (Gillespie and Macrae, 1975), and d) a temperature of 25°C appears most suitable for the organism *Moraxella* even in the presence of competing mesophyllic flora. The ratio of Gram-negative to Gram-positive organisms in the flora is approximately 70:30.

Storage Changes

Quantitative aspects

Table 3 shows changes in bacterial count during ice storage. The initial counts, ranging from 5.5×10^6 to 4.7×10^7 CFU/g, increased with the time of storage and ranged up to 2.1×10^9 CFU/g at the end of storage period. In general, the changes were similar to those previously reported (Zuberi *et al,* 1986). Table 4 shows the changes in bacterial counts during storage at 10°C. As expected, rapid increase in counts was observed after 4 days of storage as compared to 0°C (Table 1), confirming the contention that the higher the temperature, the more rapid is the growth and shorter shelf life. After 12 days storage all the samples were completely spoiled. The bacterial count reached 10^{10} CFU/g in most of the samples. The experiments demonstrate that shrimp may be stored for 4 days at 10°C without any serious loss in quality and the same level of quality may be maintained in the shrimp stored for 8-10 days in ice.

Table 5 shows the changes in the bacterial counts at room temperature (22°C-30°C). After 6 hours of storage, the count ranged from 4.3×10^6 to 9.3×10^7 CFU/g showing one log increase in most of the samples. The increase was almost the same as noticed in ice after 4-5 days. After 24 hours, a very rapid increase in counts was noticed. Most of the samples had a bacterial load of 10^{10} CFU/g (Table 3). All the samples were completely spoiled having pinkish black colour, putrid odours and slimy appearance. Similar changes were observed after 16-20 days in ice.

Qualitative aspect

Table 6 summarizes the changes in the generic composition of 7 samples of the shrimp during ice storage at 0, 2, 4, 8, 12 and 16 hours of storage. The microbial flora of fresh shrimp in order of predominance was composed of *Moraxella, Pseudomonas* Group III, *Vibrio, Micrococcus, Flavobacterium, Bacillus*, and *Pseudomonas* Group II. The percentage composition of the flora of fresh shrimp used in these experiments was found to be somewhat different from the flora reported (Zuberi *et al.* 1986) and may be attributed to the difference in sampling time. However, the behaviour of the organisms was similar as already reported (Zuberi *et al.* 1986).

The genera *Vibrio* and *Flavobacterium* disappeared and *Alteromonas* and *Pseudomonas* Group II gradually increased with storage (Table 7). *Moraxella* and *Pseudomonas* Group III dominated both in fresh shrimp and throughout the storage. These organisms being psychrotrophic in nature found favourable conditions during ice storage and multiplied at a more rapid rate than the other organisms. This is in agreement with the findings of Cobb and Vanderzant (1971) that the spoilage pattern of shrimp depends upon the initial microflora, because usually the same groups of organisms are present during spoilage as are found on freshly caught shrimp. The percentage distribution of organisms, however, alters markedly during storage and the shrimp spoils as a result of increased microbial activity. It may be seen (Table 4) that in these experiments also, the behaviour of *Micrococcus* and *Bacillus* was similar as reported earlier (Zuberi *et al.* 1986).

Table 7 summarizes the changes in the generic composition of the bacterial flora during 10°C storage. From the initial flora similar to the samples stored at °C, somewhat different flora emerged during storage as a result of elevated storage temperature. The genus *Vibrio* increased up to 4 days and then persisted throughout the storage in low proportions in contrast to ice storage, while both *Vibrio* and *Flavobacterium* disappeared during early storage. *Alteromonas putrefaciens* behaved differently if compared with ice storage. This organism appeared after 8 days of storage and persisted throughout the storage in low proportion. *Pseudomonas* Group III persisted in a higher proportion up to 12 days of storage. *Moraxella* was present throughout the storage as in a low proportion, if compared with the ice stored samples (Table 4). In contrast to the 0°C storage, at 10°C the genus *Micrococcus* gradually increased as the storage progressed and progressed and dominated the flora at the end of storage (Table 7). At the end of the storage period, the flora in order of predominance was composed of *Micrococcus, Moraxella, Pseudomonas* Group III, *Vibrio, Bacillus, Flavobacterium* and *Alteromonas.* The *Moraxella* group was replaced by the genus *Micrococcus* at 10°C storage.

Table 8 summarizes the changes in the generic composition of bacterial flora during storage at room temperature. No significant qualitative changes in the flora were observed after 6 hours storage at room temperature (22°C-30°C). Organisms initially present were observed with slight variations in percentage. However, *Vibrio* increased if present initially. Generally, the percentage of *Moraxella* decreased and *Micrococcus* increased while *Pseudomonas* Group III persisted almost in the same proportion. After 24 hours storage, mesophyllic, *Vibrio, Pseudomonas* Group III, *Bacillus* and *Micrococcus* dominated the flora and seem to play an important role in the spoilage of shrimp at room temperature.

Shrimp are an excellent medium for the growth of bacteria. Fresh shrimp normally contain a considerable number of organisms which, when given suitable conditions of elevated temperature, can multiply rapidly to a level that completely spoils the shrimp within 24 hours. One log increase in the bacterial count after 6 hours at room temperature in most of the samples is of great significance since during commercial practice in Pakistan the shrimp remain at room temperature for hours without ice at the harbour before auction which results in the loss of quality. Therefore, a major amount of the catch reaches the consumers and processing plants in an inferior condition. Rapid bacterial multiplication has been reported at elevated temperatures by other workers. Fieger *et al* (1958) reported that the bacterial count of shrimp exposed to air temperature (25°C-29°C) for 6 hours was twice that of freshly caught shrimp. However, this initial change was found to exercise a profound effect on bacterial counts compared to controls. Cobb *et al.* (1977) reported that the handling of shrimp for 3 hours at 30°C-44°C caused serious quality deterioration and, in general, the level of deterioration was comparable with the shrimp held for 6 hours at 20°C and 24 hours at 10°C.

The results of this study demonstrate that in the storage of shrimp at 0°C-10°C and 22°C-30°C the changes in initial flora of fresh samples depends upon the temperature of storage, their appearance, disappearance and changes in present composition are, however, temperature dependent. Similar findings have been reported by Matches (1982). It may be concluded that organisms which dominate and may be considered responsible for the spoilage of shrimp during storage in ice (0°C) are *Alteromonas, Moraxella, Pseudomonas* Group II and III and *Micrococcus*. At 10°C storage, more mesophyllic organisms such as *Micrococcus* persist along with the organisms already mentioned during ice storage and they spoil the shrimp earlier than the ice stored samples. The same may be said for the samples left at room temperature (22°C-30°C) where mesophyllic flora composed of *Vibrio, Pseudomonas* Group III, *Bacillus* and *Micrococcus* appear to be responsible for spoilage.

An examination of the data presented enable a number of observations to be made.

The multiplication of organisms occurs at all three storage temperatures and the rate of the growth of organisms is temperature dependent. In this study, shrimp were considered spoiled after 12 days in ice (0°C), 4-8 days at 10°C and between 6 and 24 hours at room temperature. Rapid deterioration and short shelf life of shrimp at 10°C clearly indicate that this is not an effective temperature for the storage of shrimp. The results, therefore, demonstrate the need on maintaining as low a temperature as possible during the commercial handling of shrimp. These requirements are in contrast to those found in commercial handling of our shrimp which are handled generally without ice at ambient temperatures.

Biochemical Changes

Tables 9 and 10 show the spoilage potential; bacterial counts and biochemical and organoleptic (odour, colour, texture) changes in raw and boiled inoculated shrimp, respectively. Control raw shrimp tails stored for 14 days at 0°C showed little changes organoleptically. There was no discolouration but the characteristic odour of new caught shrimp was lost and replaced by a neutral bland one. The texture was somewhat tougher due to gradual tissue dehydration. In the boiled shrimp controls, there was no off-odour but the smell of freshly boiled shrimp was absent and the texture was noticeably drier and tougher. No growth was obtained in boiled controls but three of the six raw controls had an average count of 9.2 x 10^3CFU/g, usually of *Moraxella* and *Micrococcus.* The control samples did not show any changes in pH, trimethylamine, and total volatile bases, except for the raw control, where a slight increase in total volatile bases was observed compared to the zero-hour fresh control samples. This increase, however, was negligible when compared to the inoculated samples. It was important to note that neither bacterial growth nor spoilage odours could be detected in the uninoculated boiled shrimp. Thus, all changes may be attributed to bacterial activity in inoculated shrimp samples.

Two strains of *Alteromonas putrefaciens* (formerly called *Pseudomonas putrefaciens* were used in the experiments. Based on colony characteristics, they were designated as rough (R) and smooth (S). Both strains showed similar, remarkable organoleptic and chemical changes. The pH increased from 7.0 (zero-hour fresh control) to 7.7 in inoculated raw and boiled shrimp and there were putrid odours, slimy texture and increases in trimethylamine and total volatile bases in the raw inoculated samples (Tables 1 and 2). These results are in agreement with the findings of other workers (Long and Hammer, 1941; Castell *et al.* 1949; Levin, 1968; Herbert *et al.* 1971; Shewan, 1971 and 1977; Van Spreekens, 1971 and 1977; Gilardi, 1975; Leet *et al.* 1977,

Shrimp inoculated with Pseudomonas Group II (oxidative) developed ammoniacal odours, became slimy, mostly yellow in appearance, and had increases in pH and total volatile bases, similar to reports on the spoilage potential of *Pseudomonas* groups (Shewan *et al,* 1969; Shaw and Shewan, 1968). Cobb and Vanderzant (1971) reported that shrimp inoculated with *Pseudomonas* became putrid two to three days sooner than their controls; had higher levels of water-soluble proteins, non-protein nitrogen and total volatile nitrogen than their corresponding controls, and reached a higher pH (8.0) sooner than the controls. Similar to the findings of this study, no increase in trimethylamine level was observed. Herbert *et al.* (1971) also reported off odours and flavours of naturally spoiling cod and haddock in sterile cod muscle blocks inoculated with *Pseudomonas.*

The *Moraxella* microorganisms caused no major organoleptic or chemical changes. There were no changes in pH and trimethylamine, and total volatile bases increased slightly compared to fresh and stored control samples. In the literature this group is described as nonmotile, penicillin sensitive coccobacilli that are biochemically less active than *Pseudomonas* species (Shewan *et al.* 1960, Shewan, 1971). They produced a slight spoilage odour or none (similar to Shaw and Shewan, 1968; Shewan, 1977). Their reaction patterns were predominantly negative and their spoilage potential was low.

Yellow and orange varieties of *Micrococcus* are encountered in different proportions in fresh and spoiled shrimp (Zuberi *et al.* 1985 and 1986). In our study, no noticeable organoleptic or biochemical changes were produced by these organisms in inoculated shrimp (Tables 9 and 10). Shaw and Shewan (1968) reported that many organisms present in spoiled fish play an active role in the spoilage process but when individually inoculated as pure cultures in sterile shrimp muscle, produce no spoilage characteristics. It should be noted, however, that under experimental conditions these bacteria have no other competing spoilage organisms that might cause synergetic or antibiotic effects that result in the spoilage of fish.

Although the bacterial role in fish spoilage has long been recognized, the mechanism of degradation and the extent of fish muscle protein involvement have not been elucidated. Several workers have shown that *Pseudomonas, Moraxella* and *Alteromonas* are the predominant groups contributing to spoilage.

On the basis of biochemical changes, these microorganisms may be divided into two groups. One group is composed of active spoilers such as *Alteromonas* S and R variants and *Pseudomonas* (oxidative), which produce prominent biochemical, and organoleptic changes in inoculated shrimp muscle. The second group *Pseudomonas III, Moraxella* and *Micrococcus* produce changes but not to the extent of the first group. These organisms play an important role during spoilage, at least at low ice-storage temperatures.

Vibrio species are predominant in the flora of fresh shrimp. However, due to the strict mesophyllic nature of this genus, these organisms disappeared during ice storage and could not be isolated from the spoiling shrimp. This indicates that they apparently do not contribute to spoilage at ice temperature. The role of these organisms at a higher temperature in shrimp spoilage is still to be established. Similarly, the role of *Moraxella, Pseudomonas* Group III, and *Micrococcus* in pure cultures seems to be less prominent. The complex synergic effect of these organisms remains to be evaluated.

It must be mentioned that conclusions drawn from this study should only be taken to apply to the artificial conditions imposed in the measurements of spoilage potential of some shrimp flora.

Fish spoilage is a highly complex process involving a number of organisms producing highly specific enzymes, which preferentially act only upon certain proteins or enzymes indigenous to fish muscle. A complete understanding of the specificity of enzyme activity involving fish-shellfish spoilage microorganisms could lead to methods for inhibiting or preventing microbial spoilage of fish. This subject is certainly beyond the scope of the present work. It is clear that much study is required to understand the mechanism of fish and shrimp spoilage.

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Table 1

Percent distribution of microorganisms in Karachi coastal water shrimp Jaira (*P. merguiensis)* landed at Ibrahim Hydri

Microorganisms isolated at 30°C incubation	Raw shrimp samples												
	VI*	VIII	IX	X	XI	XI	I	II	III	IV	V	VI	mean
Pseudomonas	0	41	0	19	0	0	40	0	-	20	26	9	14.09
Vibrio	62	0	27	44	61	0	0	41	-	80	0	91	36.0
Moraxella	0	0	42	0	23	0	43	26	-	0	0	0	12.18
Micrococcus	20	18	11	0	16	58	17	33	-	0	0	0	15.72
Staphylococcus	0	0	0	0	0	0	0	0	-	0	0	0	0.0
Bacillus	18	0	3	0	0	20	0	0	-	0	20	0	5.5
Flavobacterium	0	41	0	0	0	22	0	0	-	0	54	0	10.63
Acinetobacter	0	0	0	22	0	0	0	0	-	0	0	0	1.63
Alteromonas	0	0	18	0	0	0	0	0	-	0	0	0	2.0
Unidentified	0	0	0	15	0	0	0	0	-	0	0	0	1.36

*The microbial counts per sample ranged from 3.2×10^5 to 4.7×10^7CFU/g (Median 2.6×10^6CFU/g.)

Table 2 Percent distribution of microorganisms in Karachi coastal water shrimp Jaira *(P. merguiensis)* landed at Ibrahim Hydri

Microorganisms isolated at 25°C incubation	Raw shrimp samples											
	VI*	VIII	IX	X	XI	XI	XII	I	II	III	IV	V	mean
Pseudomonas	0	22	23	0	20	41	34	13	25	20	0	0	16.6
Vibrio	51	43	35	67	20	0	0	0	0	0	64	76	29.7
Moraxella	0	0	33	0	17	36	20	43	39	52	0	0	20.0
Micrococcus	0	28	9	17	16	0	41	32	16	12	26	21	18.2
Bacillus	15	0	0	16	8	0	0	0	0	10	3	3	54.3
Flavobacterium	34	7	0	0	19	0	0	12	0	0	0	0	6.0
Acinetobacter	0	0	0	0	0	0	0	0	12	0	0	0	1.3
Alteromonas	0	0	0	0	0	23	5	0	9	0	0	0	4.0

The microbial counts per gram sample ranged from 5.3 x 10^5 to 8.3 x 0^7CFU/g (Median 3.1 x 10^6 CFU/g)
* Months of the year

Table 3 Changes in the bacterial count of shrimp Jaira *(Penaeus merguiensis)* stored at 0°C

Experiment No.	TPC/g	Days of Storage				
	0	2	4	8	12	16
1	6.3×10^5	*	4.2×10^5	1.4×10^6	1.7×10^7	7.8×10^9
2	1.2×10^6	8.0×10^6	1.5×10^7	1.5×10^7	9.4×10^8	9.9×10^8
3	9.9×10^6	1.7×10^7	3.4×10^7	3.4×10^7	3.1×10^{10}	7.3×10^9
4	9.8×10^6	2.1×10^7	1.4×10^7	1.4×10^7	7.8×10^8	4.5×10^9
5	4.7×10^7	7.1×10^7	9.6×10^7	9.6×10^7	5.0×10^9	3.2×10^9
6	5.5×10^5	6.2×10^5	7.8×10^6	7.8×10^6	2.6×10^8	2.1×10^8
7	1.4×10^6	8.5×10^5	7.2×10^5	7.2×10^5	6.3×10^8	1.1×10^9

*Not done

Table 4 Changes in the bacterial count of shrimp Jaira (*Penaeus merguiensis*) stored at 10°C

Experiment No.	TPC/g	Days of Storage				
	0	2	4	8	12	16
1	6.3×10^{5}	*	9.6×10^{5}	1.0×10^{7}	4.3×10^{8}	2.5×10^{10}
2	1.2×10^{6}	2.1×10^{7}	1.3×10^{8}	1.3×10^{9}	1.5×10^{9}	3.9×10^{9}
3	9.9×10^{6}	9.3×10^{7}	9.7×10^{7}	7.2×10^{8}	2.6×10^{9}	4.0×10^{8}
4	9.8×10^{6}	2.2×10^{6}	4.6×10^{7}	8.0×10^{8}	1.1×10^{9}	2.7×10^{9}
5	4.7×10^{7}	3.8×10^{7}	5.8×10^{9}	3.4×10^{9}	5.5×10^{9}	5.2×10^{9}
6	5.5×10^{5}	3.2×10^{7}	6.6×10^{7}	5.0×10^{8}	3.0×10^{8}	2.5×10^{8}
7	1.4×10^{6}	8.5×10^{5}	4.6×10^{8}	1.7×10^{9}	3.7×10^{9}	8.6×10^{8}

*Not done

Table 5 Changes in the bacterial count of shrimp Jaira (*Penaeus merguiensis*) stored at room temperature (22-30°)

Experiment No.	TPC/g	Time storage	
	0 hr.	6 hr.	24 hr.
1	2.3×10^{6}	1.5×10^{7}	3.8×10^{10}
2	6.3×10^{5}	4.3×10^{6}	1.4×10^{9}
3	1.2×10^{6}	7.6×10^{6}	1.2×10^{9}
4	9.9×10^{6}	7.2×10^{7}	2.3×10^{10}
5	9.8×10^{6}	5.3×10^{7}	6.4×10^{10}
6	4.7×10^{7}	9.3×10^{7}	1.6×10^{9}
7	5.5×10^{5}	3.3×10^{7}	5.9×10^{10}

Table 6 Summary of changes in percentage distribution of bacterial flora of shrimp Jaira *(Penaeus merguiensis)* during storage at 0°C

Days of storage	Pseudomonas	Pseudomonas	Vibrio	Moraxella	Alteromonas	Flavobacterium	Micrococcus	Bacillus
	Group II	Group III						
0	2.9	27.15	23.2	32.0	0.0	4.45	6.2	3.8
2	0.0	33.6	8.6	26.3	1.6	13.47	16.0	0.28
4	0.0	56.6	0.0	24.0	0.0	9.5	9.0	0.83
8	5.3	48.0	0.0	28.5	4.1	0.0	13.0	1.6
12	16.8	32.2	0.0	24.1	18.1	0.0	8.6	0.0
16	18.2	28.0	0.0	14.2	28.4	0.0	11.2	0.0

Table 7 Summary of changes in percentage distribution of bacterial flora of shrimp Jaira *(Penaeus merguiensis)* during storage at 10°C

Days of storage	Pseudomonas	Pseudomonas	Vibrio	Moraxella	Alteromonas	Flavobacterium	Micrococcus	Bacillus
	Group II	Group III						
0	2.9	27.1	23.2	32.10	0.0	4.45	6.2	3.8
2	3.0	21.8	36.1	10.5	2.0	0.0	20.3	6.1
4	6.0	28.8	32.3	11.1	0.0	1.3	16.8	3.5
8	8.8	27.1	14.1	5.5	7.5	0.0	25.6	10.8
12	0.0	27.0	13.0	20.8	3.3	2.1	31.6	8.0
16	0.0	10.6	10.0	12.6	3.0	4.0	49.4	5.8

Table 8 Summary of changes in percentage distribution of bacterial flora of shrimp Jaira *(Penaeus merguiensis)* during storage at room temperature (11-30°C)

Storage time	Pseudomonas	Pseudomonas	Vibrio	Moraxella	Micrococcus	Flavobacterium	Bacillus
	Group II	Group III					
fresh	2.9	27.15	23.2	32.0	6.2	6.2	3.8
6 hrs.	0.0	25.0	36.6	20.6	13.2	13.2	2.6
24 hrs.	0.0	24.4	36.4	0.0	7.40	7.4	21.8

Table 9 Spoilage potential of pure bacterial cultures on sterile muscle substrate of raw Jaira shrimp (*Penaeus merguiensis*) stored at 10°C for 14 days

Culture species	TPC/g	pH	TMA* (mg/100g)	TVB** (mg/100g)	Odour	Colour	Texture
Pseudomonas Group II	10^9	7.6	0.9	13.6	amononiac	pale yellow	soft slimy
Pseudomonas Group III	10^8	7.0	0	4.5	stale	slight change	firm
Alteromonas Putrefaciens S	10^9	7.7	13.7	40.3	putrid	dirty	very soft
Alteromonas Putrefaciens R	10^9	7.7	17.9	41.9	putrid	dirty	very soft
Moraxella	10^8	7.1	0	5.3	musty	slight	firm
Micrococci							
- yellow pigmented	10^7	7.0	0	7.0	musty	slight change	firm
- orange pigmented	10^7	7.1	0	6.6	musty	slight change	firm
Control-fresh shrimp	0-10^3f	7.1	0	1.2	fresh	normal	normal
Control-stored shrimp	0-10^3	7.1	0	3.1	fresh odour lost	slight	tough

* = trimethylamine ** = total volatile bases f = in three samples

Table 10 Spoilage potential of pure bacterial cultures on sterile muscle substrate of raw Jaira shrimp (*Penaeus merguiensis*) stored at 0°C for 14 days

Culture species	TPC/g	pH	TMA* (mg/100g)	TVB** (mg/100g)	Odour	Colour	Texture
Pseudomonas Group II	10^9	8.0	2.2	15.5	amononical	pale yellow	soft slimy
Pseudomonas Group III	10^8	7.1	0	9.0	musty	slight change	firm
Alteromonas Putrefaciens S	10^9	7.7	13.5	40.3	putrid	dirty	very soft
Alteromonas Putrefaciens R	10^9	7.7	12.0	42.6	putrid	dirty	very soft
Moraxella	10^8	7.3	2.8	12.1	musty		firm
Micrococci							
- yellow pigmented	10^7	7.0	2.0	12.0	musty	slight change	firm
- orange pigmented	10^7	7.2	3.2	15.0	musty	slight change	firm
Control-fresh shrimp	0	7.1	1.1	9.2	fresh	normal	normal
Control-stored shrimp	0	7.1	1.0	8.4	fresh odour lost	slight change	tough

* trimethylamine ** total volatile bases

COMBINATION EFFECTS OF IRRADIATION, PACKAGING AND N_2-GAS FOR SHELF-LIFE EXTENSION OF SHRIMP

by

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ABSTRACT

The influence of different parameters on the shelf-life of cooked shrimp experiments was evaluated. Irradiation treatments included shrimp (peeled and unpeeled) at different temperature (deep frozen at -35°C and chilled at 5°C) and using different packing materials ethylene vinylalcohol copolymer polypropylene and polyethylene (EG-Q) and cellophane (CP). To reduce the oxidation effects, the experiments were performed under nitrogen gas.

Irradiation was done with gamma rays emitted by a cobalt-60 source of 5.55×15^{15} Bq (150 KCi) at a dose rate of 6 kGy. per hour. The usual irradiation doses of 1.5 and 3 kGy. were applied as well as a range of 0.5, 1, 2, 3, 4, 5, 6 and 8 kGy.

The experiments confirmed that the radiation sensitivity of microorganisms like coliforms and aerobic microorganisms and enterobacteriaceae is temperature dependent.

The peroxide value was also temperature dependent and was affected by the packaging material.

The TMA-N value was not significantly influenced by the applied radiation doses, shrimp irradiated at chilled conditions showed off-flavours at 1.5 kGy. Off-flavours could be detected at a dose of 3 kGy when the shrimp was irradiated in deep frozen condition. No difference was observed on the radiation effects of peeled and unpeeled shrimp.

INTRODUCTION

Since 1985 farmed shrimp especially tiger (*P. monodon)* was introduced for frozen shrimp export. Unfortunately, shrimp was rejected because of high bacterial count and also Salmonella contamination, especially by the FDA, USA. Salmonella contamination on farmed shrimp was detected even when good manufacturing practice had been applied, as previously reported by Rattagool (1986). One disadvantage noted with irradiation was the formation of off-odours which developed on shrimp, especially those irradiated at higher doses, off-odours follow treatment at 3 kGy in cooked shrimp when irradiated under chilled condition. Higher doses can be applied if shrimp are irradiated frozen.

OBJECTIVES

1. To extend the shelf-life of frozen shrimp and cooked shrimp.
2. To study the chemical and microorganism change during storage.
3. To find the radiation dose to eliminate pathogenic microorganisms.

4. To investigate the radiation dose, that causes off-flavour, of cooked or uncooked shrimp under frozen or chilled condition.

MATERIALS AND METHODS

Chemical analysis

Trimethylamine nitrogen (TMA-N) was estimated by the A.O.A.C. method (Horowitz, 1980).

Peroxide values, indicating the state to which lipid has oxidised, were measured by the method of Folch (in Horowitz 1980). They are expressed in mulli-equivalents of peroxide per kilo of sample.

Microbiological analysis

Total bacterial count (TVC/gm) was carried out following the Bacteriological Analytical Manual (BAM) for food, FDA (1976). Nutrient agar used for standard plate counts but .2% glucose, 5% yeast extract and .2% K_2HPO_4 were added (Ito, H. modification) and Coliforms were enumerated on MacConkey agar, and incubated at 30°C, overnight and two days.

Sample preparations

Headless and shell on frozen shrimp (size 26-30 per pound) from South East Asia was used for three treatments.

1. Cooked and peeled shrimp

Shrimp was cooked at 100°C for 3 minutes in 1% brine solution. 5 gm of each sample was packed into EG-Q and cellophane (CP) bags with nitrogen flushing. The samples in EG-Q and CP were irradiated at 0.5, 1, 2, 3, 4, 5, 6 and 8 kGy under frozen conditions on both treatments. POV was determined on the first treatment but POV, TMA and TVC were analyzed on the second treatment.

RESULTS AND DISCUSSIONS

Treatment 1. (irradiated frozen shrimp)

It was found that POV on the treated shrimp in EG-Q bag with N_2-gas irradiated (at 0, 1, 2, 3, 4, 5, 6 and 8 kGy) under chilled conditions, increased rapidly at higher radiation doses. It also slightly increased on shrimp using the same treatment, but irradiated under frozen conditions (Fig. I).

The TVC of frozen shrimp at higher irradiation doses (>1 kGy) showed that the microorganisms were more resistant compared with those in chilled shrimp (Fig. II).

The same reaction was observed on coliforms but these microorganisms seem to be very sensitive even at lower radiation doses (1 kGy). Off odour was also investigated by sensory evaluation. It was first detected at a radiation dose of 1 kGy and would be rejected in chilled shrimp at 3 kGy and at 5 kGy for frozen shrimp.

Treatment II (irradiated cooked-shell on-shrimp)

POV was investigated between EG-Q and CP packing on the irradiated shrimp (at 1, 1.5 and 3 kGy) under frozen condition. It was found that POV was higher at 3 kGy than 1.5 kGy and also higher than 0 kGy (Figure III).

On irradiated shrimp CP. packaging gave a higher POV at the higher radiation doses (3 kGy) which was similar to shrimp in EG-Q bags.

The POV increases as the radiation dose increases in both (EG-Q) and (CP). However, samples in EG-Q packing gave, at lower POV. than samples in CP packing. Microorganisms did decrease while the radiation doses were increased giving a D_{10} value of 2.79 kGy.

Red colonies were identified as *Deonoccus sp.* (Ito, H. *et al.*, 1986). They were found at higher doses. Storage of irradiated cooked and peeled shrimp at 0, 1.5 and 3.0 kGy. for 0, 3, 6, 9 and 12 days under 5°C storage was investigated.

Cooked peeled shrimp in both packs irradiated at 1.5 and 3 kGy frozen, gave low counts (10^2 cfu) even at 0 kGy. However, bacterial numbers were slightly decreased for the cellophane packing especially at higher radiation dose.

Table 2 shows that the development of POV is influenced by a combination of factors, oxygen permeability of the packaging material, radiation dose and storage time. In the shrimp packed in N_2 under frozen conditions the POV develops faster with cellophane. POV also increases with the higher radiation doses for both packaging materials.

Table 3 POV developed more slowly at the higher radiation doses after 5 days storage. TVC on cooked and peeled shrimp is easy to eliminate at 3 kGy because it was low at day 0. TMA also changed very little with the radiation doses.

Table 1. Change in microorganisms on cooked (shell-on) frozen shrimp packed in EG-Q and cellophane with N_2-gas then irradiated frozen (dry-ice) at 0, 1.5 and 3.0 kGy, and stored at 5°C for 12 days

Storage (days)	TVC (cell/gm					
	EG-Q film			Cellophane		
	0 kGy	1.5 kGy	3.0 kGy	0 kGy	1.5 kGy	3.0 kGy
0	2.25×10^2	2.5×10^2	N.D.	2.25×10^2	3.75×10^2	N.D.
3	1.4×10^2	7.5×10^2	1.5×10^2	4.25×10^2	1.0×10^2	5.0×10^1
6	2.5×10^2	3.3×10^1	3.3×10^1	6.2×10^2	6.7×10^1	3.3×10^1
9	2.5×10^2	2.6×10^1	2.5×10^1	3.25×10^2	N.D.	N.D.
12	1.75×10^2	1.5×10^1	5.1×10^1	1.5×10^2	1.5×10^1	5.0×10^1

Table 2 Peroxide value changes in frozen cooked (peeled) shrimp which was packed into EG-Q film with N_2-gas then irradiated frozen at 0, 1.5 and 3.0 kGy and stored at 5°C for 12 days

Packaging materials	Storage (days)	POV (meq/kg)		
		0 kGy	1.5 kGy	3.0 kGy
EG-Q	0 3 6 9 12	17.640 44.370 51.462 N.A. 75.641	13.259 50.939 56.876 N.A. 99.851	17.443 58.799 62.329 N.A. 97.902
Cellophane	0 3 6 9 12	15.910 46.752 68.545 N.A. N.A.	20.999 56.023 87.545 N.A. 102.461	26.540 81.302 112.332 N.A. 176.053

N.A. = not available sample

Table 3 Cooked and peeled frozen shrimp were packed in EG-Q bags then irradiated at 1, 3, 5 and 8 kGy frozen. TMA-N, peroxide value and total viable aerobic microorganisms were determined at 0 and 5 days after 5°C storage.

Dose (kGy)	P.V. (meq/kg)		TVC/gm		TMA-N (mgN%)	
	0 day	5 days	0 day	5 days	0 day	5 days
0	29.059	31.625	$3.0x10^2$	$6.25x10^2$	3.050	1.742
1	30.996	31.360	$2.0x10^2$	$3.0x10^2$	2.915	1.921
3	40.464	38.360	$1.25x10^2$	0	2.875	1.805
5	42.702	45.614	0	0	2.464	1.160
8	41.467	65.674	0	0	2.690	0.962

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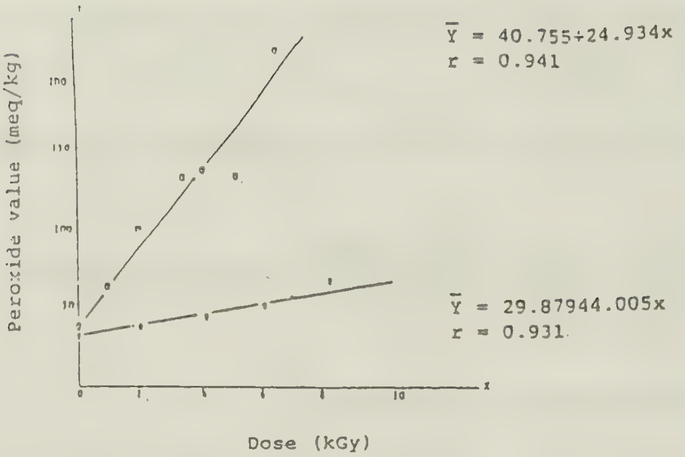

Fig. I Frozen headless shell-on shrimp packed in EG-Q with N_2-gas then irradiated at 0, 1, 2, 3, 4, 5, 6 and 8 kGy with a radiation rate at 6 kGy per hour.

● = irradiated frozen
O = irradiated unfrozen

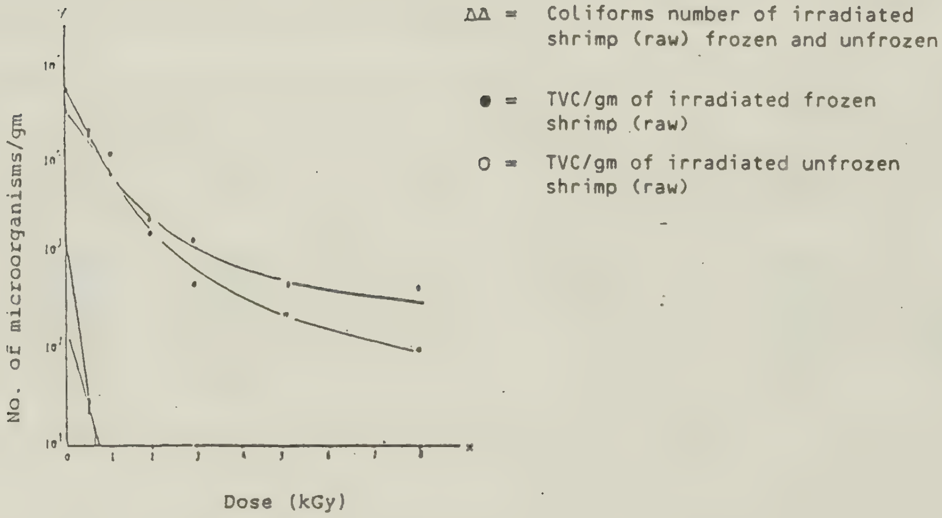

Fig. II Changes in total viable aerobic count (TVC/gm) and Coliform number when radiation dose is increased.

●▲ = irradiated frozen
OΔ = irradiated unfrozen

● = 0 kGy., $\bar{Y} = 23.496+4.53x$, $r = 0.972$
■ = 1.5 kGy., $\bar{Y} = 19.636+6.76x$, $r = 0.976$
▲ = 3.0 kGy., $\bar{Y} = 26.87 +6.14x$, $r = 0.956$
○ = 0 kGy., $\bar{Y} = 17.418+8.77x$, $r = 0.950$
□ = 1.5 kGy., $\bar{Y} = 11.35 +15.48x$, $r = 0.988$
△ = 3.0 kGy., $\bar{Y} = 35.699+12.07x$, $r = 0.991$

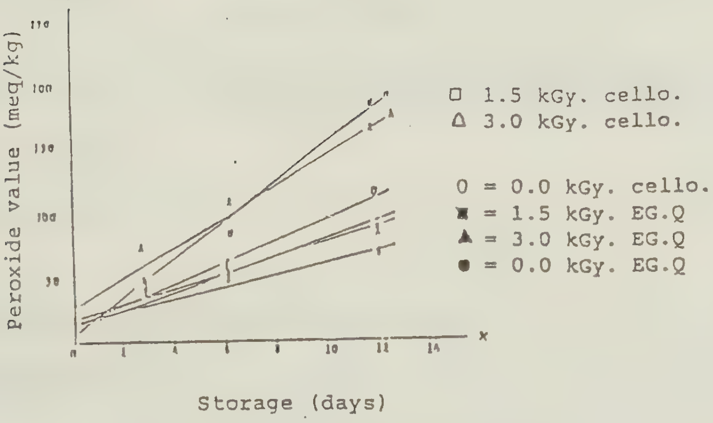

Fig. III Frozen cooked shrimp packed in EG-Q and cellophane bags with N_2-gas then irradiated frozen at 1.5, 3.0 kGy and stored at 5°C for 12 days.

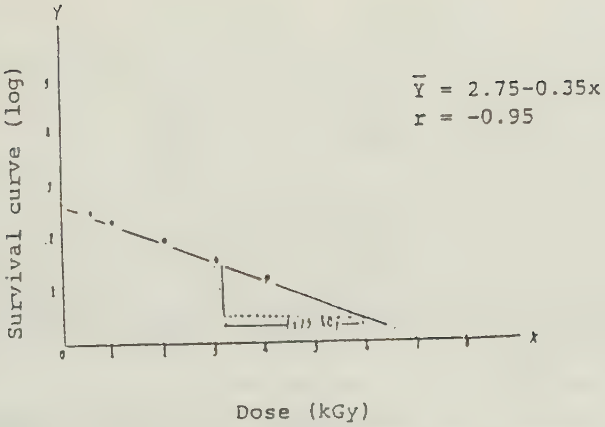

Fig. IV Changes in microorganisms after frozen cooked shrimps (shell-on) were packed in EG-Q bags with N_2-gas, then irradiated frozen at 0, 0.5 1, 2, 3, 4, 5, 6 and 8 kGy.

EFFECT OF CHILLED WATER STORAGE ON INDOLE PRODUCTION AND WATER CONTENT IN BRACKISHWATER SHRIMPS

by

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ABSTRACT

A study of the effect of handling shrimps using a chilled water system (CWS) on indole production, water content and bacterial counts was performed in August 1990. All parameters were examined at 24 hour intervals. CWS involved holding shrimps in barrels which contained a mixture of crushed ice and water.

During storage of 104 hours, the moisture content of shrimps increased from 83.24% to 85.70% (an increase of 2.46%). Initial content of indole in the shrimps was 10.102 μ/100g and increased to 20.181 μg/100g at the end of observation (an increase of 10.79 μg/100g). So, the shrimp was still of first quality (<25μ/100).

Total plate counts in the shrimp, at incubation temperature of 20°C ranged between 5.21 and 5.96 log (an increase of 0.71) during 104 hours of handling.

INTRODUCTION

Shrimp is the most important export fishery commodity for Indonesia. This product is also distributed in local markets, in particular supermarkets, restaurants and hotels.

Handling shrimps using chilled water system (CWS) and crushed ice is common during storage and transportation. Some shrimps harvested or caught are not directly sold to processing units, but they pass through a long chain. The chain is started from ponds, collection places and processing units. Some farmers sell their fresh shrimps directly to processing units. Buying managers of the processing units also visit the ponds to make a bargain. Remote location of the ponds, and a limited transport service are the other difficulties encountered in shrimps transport.

To maintain freshness in shrimps, use of ice after harvesting is recommended. Handling shrimps using chilling water is done by holding the shrimps in barrels or boxes containing ice and water. The ratio of shrimp to ice is normally 2:1, depending on length of storage and distance (Peterson, 1974). This system results in less physical damage to the shrimps. It is suspected that shrimp weight increases during storage and transportation. So, from the buyer's point of view, the increasing weight is a disadvantage of CSW.

Indole is currently used by the US Food and Drug Administration to validate the sensory evaluation of shrimp decomposition. Class 1 is passable and possesses no definite odour identifiable as decomposition, class 2 is the first stage of readily perceptible decomposition to the experienced examiner and class 3 possesses a strong odour of decomposition. Corresponding indole levels have been established at less than 25 μg/100g for class 1, and >25 μg/100g for classes 2 and 3 (Chang, *et al,* 1983.

MATERIALS AND METHODS

Material

Fresh shrimps were directly collected from a brackishwater pond located in Tanggerang, West Java. The shrimps were transported to the National Centre for Fish Quality Control and Processing Development, Jakarta, by car. During transportation the shrimps were put in a box with addition of crushed ice.

Handling shrimps

The shrimps were put in boxes containing a mixture of ice and water. The ratio of water to ice was 1:2. The shrimp were withdrawn at 12 intervals and subject to laboratory analysis.

Experimental design

A randomized design was used in which the period of handling was regarded as a treatment.

The treatments included 0 hours, 20 hours, 32 hours, 44 hours, 56 hours, 68 hours, 80 hours, 92 hours and 104 hours. Each treatment had 3 replicates.

Laboratory analysis

Indole Determination: Determination of indole in shrimp was by a method developed by Chenk and Finne (1981). Procedure of the analysis is outlined as follows:

1. 40 g of shrimp flesh was mixed with 80 ml TCA and blended for one minute.
2. 80 ml petroleum benzene was added to the mixture and blended in a container chilled with ice for one minute.
3. Homogenate was centrifuged for one minute at 4000 rpm and filtered using a No. 1 Whatman paper.
4. Petroleum benzene containing indole was separated from TCA by a separating funnel.
5. Indole extract was mixed with 5 ml Fehrlich's solution and shaken hard for one minute and left to stand to obtain two layers.
6. The lower layer is the indole extract and its absorbance is determined by a spectrophotometry at 570 nm.
7. A standard curve of indole was produced by preparing a serial dilution of five points.

Moisture content: Determination of moisture content in the shrimps was by AOAC (1984), using a gravimetric method. 5 g of shrimp flesh was put in a porcelain crucible and heated in oven at 100°C for 5 hours. The crucible containing ash was placed in a desiccator and weighed to obtain dry weight. The moisture content was calculated as follows:

$$\frac{\text{Wet weight - dry weight}}{\text{wet weight}} \times 100\%$$

Total plate counts

TPC analysis was according to BAM (1984). 25g of shrimp was put in a sterile plastic bag containing 225 ml BFP and blended in a stomacher for two minutes. A serial dilution was made until 10^{-5} and 0.1 ml PCA. All plates were incubated at 20°C for 24 to 48 hours. Plates containing 30-300 colonies were calculated.

RESULTS AND DISCUSSION

Indole content in shrimps

Results of indole production in shrimps handled using CWS for 104 hours were presented in Figure 1. Initial indole content was 10.10 ug/100g. Percentage increases of indole at t1, t2, t3, t4, t5, t6, t7 and t8 were 21.45%, 35.69%, 40.39%, 64.06%, 79.04%, 94.15% and 99.77% respectively. Indole content at the end of handling (t8) was 20.18 ug/100g. So, the shrimp stored in CWS for four days and eight hours was still in the range of first class (less than 25 μg/100g). The time taken for harvesting shrimps and transporting them into processing units was normally between one and three days, depending on distance and location of ponds. This suggests that handling shrimp using this method can maintain the shrimps in the first quality, as far as indole content is concerned. Soeparjo (1988) reported that indole content in the same shrimps stored at ambient temperature for four days increased 650%. However, the increase was only 71% in a chill room for the same period of handling.

Statistically, there was a significant correlation between the content of indole and period of handling. The linear regression between them was $Y = 0.1001 X + 9.649$.

Moisture content

The results of moisture content in the shrimp stored in CWS for 104 hours are shown in Figure 2. In general, the moisture content in the shrimps increased in parallel with period of storage. The longer incubation, the higher moisture content. Statistically, moisture content correlated significantly to period of storage. The linear regression was $Y=0.0031 X + 81.883$.

The initial moisture content was 83.33% and increased to 85.70% at the end of observation (at 104 hours). So there was a total increase of moisture content divided into three stages. First stage ranged between t0 and t3 in which shrimp were able to absorb a relatively high quantity. In this stage, shrimps were characterized by having high freshness and high elasticity and strong texture, but the available cavity in the shrimps was relatively large. The increase of water content was 1.77%.

The second stage spanned from t1 to t6. In this stage, the amount of water absorbed was very little and the increase was only 0.76%.

The third stage ranged between t7 and t8. In this stage, shrimp flesh started to soften. Hence, shrimps were able to absorb more water and the increase of water content was 1.85%. Movement of water as chilling medium into shrimp flesh is due to osmosis. This water would expand the volume, but it is not a component of shrimps (Winarno, 1988).

It is concluded that the longer period of storage, the higher moisture content became. Statistically, moisture content significantly correlated to period of storage. The linear correlation and regression between them was formulated as follows: $Y = 0.031 X + 81.883$.

Total plate counts

Total microbial contaminant on the shrimps was determined at 20°C of incubation for 48 hours. Numbers of bacteria in the shrimps stored by CWS for 104 hours are shown in Figure 3. The number fluctuated from 5.22 log (at t0) to 5.96 (at t8). At t1 (20 hours) the number increased to 5.83 and sharply decreased at t2 was only psychrophilic bacteria could grow under these conditions. From this time, the number gradually increased again and reached the peak at the end of storage (t8). At this stage, texture of the shrimps began to soften. This condition allowed bacteria to grow.

CONCLUSION

1. Storage of shrimp in CWS resulted in increase of the moisture content which correlated significantly to period of storage. The linear correlation and regression was $Y=0.031 X + 81.883$.

2. At the beginning of storage, shrimp contained 10.10 μg/100g of indole. At the end (104 hours) the content increased to 20.18 μg/100g at which shrimps were still of the first class. Indole

content correlated significantly to period of storage. The linear correlation and regression was $Y = 0.100\ X + 9.649$.

3. The initial count on the shrimps was high (5.21 log). After 104 hours storage, it increased to 5.83 log.

CHLORINATION OF SHRIMPS BY MACHINE WASHING

by

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DGF, Jakarta

ABSTRACT

Chlorination is an important method for reducing bacterial loads in products like frozen shrimp. The study investigated the effectiveness of chlorination by different methods and concentrations of chlorine. The investigation was undertaken in one of the frozen shrimp factories in Jakarta. The work investigated the effect of different methods of washing shrimp in reducing microbial content. The two methods were manual washing and machine washing. Each method was applied to 5 treatments including raw material as a control, washing with unchlorinated water, 10 ppm and 20 ppm chlorinated water and 20 ppm chlorinated water with rinsing.

The effectiveness of the chlorination method was monitored by determination of Total Plate Count (TPC), Coliforms, *Escherichia coli, Salmonella* and organoleptic testing.

The use of a washing machine for washing shrimp gave more consistent results in reducing microbial contamination that manual washing. *Salmonella* and *Escherichia* coli were not found since the raw materials were free from them. However, the average of Coliforms varied between 23 and 461 MPN/g.

The effect of washing in reducing bacterial contamination was significant particularly on Total Plate Count and Coliforms.

INTRODUCTION

More than 35 frozen shrimp factories are located in Jakarta. Their raw material is marine and brackishwater shrimp which respectively are supplied by fishermen and farmers. Marine shrimp landings recently decreased due to reduction of shrimp boats, in particular trawlers. In 1980, the Indonesian government banned the use of bottom trawlers which was thought as a cause of marine environment destruction. To anticipate this regulation, the government encouraged farmers to build new brackishwater ponds and introduced semi-intensive or intensive brackishwater culture for shrimps (*Penaeus monodon*). This succeeded in increasing production of shrimp. Brackishwater shrimp normally have higher freshness than marine shrimp, when both type of shrimp arrive at processing units, due to their differences of period of transportation and chain of collection. Generally, quality of fresh shrimp as a raw material varies between fishermen and farmers. Hence a standard handling practice should be introduced. Use of chlorine, during the washing stages, is recommended to reduce bacterial loads which may occur during handling at sea, transportation and collection. Rejection of seafood products, in particular shrimps, has been reported due to decomposition, *Salmonella* and the use of unacceptable additives or preservatives. Chlorine is recognized as a safe chemical and is still widely used in food industries as a sanitizing agent for washing food products and equipment. Banwart (1980) reviewed the literature and proposed three possible actions of chlorine. First, the action of chlorine involves the penetration of the cell. It reacts with cellular protoplasm which causes inactivation. Secondly, the chlorine reacts with key enzyme systems and prevents normal respiration. These reactions may involve oxidation or denaturation of protein or damage to SH-groups of essential enzymes. Thirdly, cell membranes are altered or disrupted by chlorine. This allows diffusion of cell contents out of the cell or toxic materials into the cell.

Most studies involving the use of chlorine in the food industries are concerned with the bactericidal properties of HOCL and aimed to increase the quality of Food (Johnston *et al,* 1983). Chlorine is often used in the form of sodium hypochlorite. It preferentially inhibits polymerase deficient bacteria. Polymerase I is active in the repair of damaged DNA (Banwart, 1980).

MATERIAL AND METHOD

Materials

Shrimp *(Penaeus monodon)* used throughout the trial were collected from brackishwater ponds located along the west Java coast. The raw materials were prepared by the factory and classified as first class shrimp.

Chlorine used was in the form of sodium hypochlorite which is normally used in Indonesian frozen shrimp factories. Active chlorine content was found to be 19%. Amounts of sodium hypochlorite used in the washing stages were based on this active chlorine.

Crushed ice was added to water and this chilled water was used for washing shrimps. Temperature of shrimp was maintained at less than 10°C. Ratio of shrimp to ice was 1:2.

Experiment design

The experiment involved two trials as follows:

i) Shrimps were manually washed, using equipment such as plastic boxes, trays, etc.

ii) Shrimps were washed by machine.

Each trial had four treatments (samples):

1. Raw materials (control
2. Shrimps washed with tap water with no chlorine
3. Shrimps washed with 10 ppm chlorinated water
4. Shrimps washed with 20 ppm chlorinated water

Parameter of analysis

Microbial test

1. Total plate counts (TPC)
2. *Escherichia coli*
3. *Salmonella*

Chlorine residue

Sensory test

Sensory test employed 6 standard panellists.

Analysis of data

Data collected, in particular TPC, were statistically analyzed using analysis of variance (Anova). To investigate differences amongst treatments (samples) on each trial, the least significant difference (LSD) was used.

RESULT AND DISCUSSION

Trial on shrimps washed manually

Washing significantly reduced bacterial load of shrimps. Similarly, shrimps washed with both 10 ppm and 20 ppm chlorinated water had significantly reduced total numbers of bacteria. However, regarding total bacterial numbers, these samples were not better than shrimp washed with unchlorinated water, since there were no differences between shrimps treated with chlorine and shrimp with no chlorine.

Raw shrimp coliform content varied between replication. Average coliform contaminant on the raw sample was 122 (MPN/g). Surprisingly, shrimp washed with 20 ppm chlorinated water contained higher coliforms than shrimp washed with 10 ppm. Total bacterial contamination of shrimp subjected to 20 ppm chlorinated water-washing was higher than that of shrimp washed with 10 ppm. Samples subjected to washing (with or without chlorine) contained lower coliform compared to the raw shrimps. All samples were free from *Escherichia coli* (MPN/g). This means that shrimps might be caught from clean water and handled properly during transportation and at collection places. It is concluded that washing with or without chlorine reduced coliform contamination of shrimp. This confirmed that the sample was not homogenous.

The result of organoleptic tests on shrimps washed manually is shown in Table 1. In the test, panellists observed texture, appearance and flavour of the samples. All samples had no significant differences in sensory value. Average values of the samples were more than 5.5 (acceptable limit). This means that the samples were still of high freshness and met the standards for seafood in Indonesia and other countries.

Trial on shrimps washed with chlorinated water using a washing machine

Results of the trial are shown in Table 2. Total bacterial counts on raw shrimp still meet the standards and criteria of seafood in many countries. The average bacterial count was log 5.2. After chlorine treatment the bacterial counts of the shrimps significantly decreased. LSD revealed that bacterial counts of the shrimps washed with chlorinated water were significantly lower than that of shrimps washed with water. This finding is contrary to the result on use of chlorine with manual washing. This significant effect may be due to longer contact of the shrimps with chlorinated water.

In this trial, washing shrimps with unchlorinated or chlorinated water significantly reduced coliforms. Coliforms in shrimp treated with chlorinated water were lower. However, shrimp treated with 20 ppm contained higher coliforms than that of 10 ppm of chlorine. No samples contained *Escherichia coli* (MPN-g).

The result of sensory evaluation is shown in Table 2. Like the results on the previous trial, all samples met standards for seafood, since the average sensory values were more than 5.5 which is an acceptable limit. There were statistically significant differences between samples.

CONCLUSION AND FURTHER WORK

1. Washing shrimps with or without chlorine was able to reduce bacterial contamination, in particular TPC and coliform. As far as coliforms are concerned, the effectiveness of 10 ppm and 20 ppm chlorine was not different. In general, microbial quality of the shrimp washed with chlorinated water was better.

2. The numbers of initial load of bacteria affect the effectiveness of chlorine.

3. Decrease of microbial contamination in shrimp washed with the washing machine was more consistent.

4. Analysis of chlorine residues on shrimps may be of importance and an applicable method of chlorine analysis should be investigated as soon as possible.

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Table 1 Microbial contamination and sensory values of shrimp washed manually

Sample	Replication	TCP/gr (log)	Coliform/MPN	E. coli	Sensory
Raw material	1	6.00	7	< 3	7.19
	2	6.48	150	< 3	
	3	6.36	210	< 3	
		6.28	122		
Shrimp washed with no chlorine	1	5.11	150	< 3	7.28
	2	5.70	9	< 3	7.56
	3	4.68	93	< 3	6.94
		5.16	84		7.26
Shrimp washed with 10 ppm chlorine	1	4.62	11	< 3	7.05
	2	4.25	15	< 3	7.33
	3	4.47	43	< 3	7.05
		4.45	23		7.14
Shrimp washed with 20 PPM chlorine	1	4.84	93	< 3	7.08
	2	4.45	43	< 3	6.95
	3	4.86	43	< 3	6.92
		4.71	59		6.98

Table 2 Microbial contamination and sensory values of shrimp washed by machine

Sample	Replication	TCP/gr (log)	Coliform/MPN	E. coli	Sensory
Raw material	1	6.00	240	< 3	7
	2	6.48	150	< 3	
	3	6.36	120	< 3	
		6.28	170		
Shrimp washed with no chlorine	1	4.85	7	< 3	7.22
	2	4.86	93	< 3	7.39
	3	4.90	240	< 3	6.97
		4.87	119		7.19
Shrimp washed with 10 ppm (chlorine)	1	4.62	11	< 3	7.22
	2	4.25	15	< 3	7.06
	3	4.47	43	< 3	7.14
		4.45	23		7.14
Shrimp washed with 20 ppm (chlorine)	1	4.30	43	< 3	7.03
	2	4.32	43	< 3	6.72
	3	4.25	43	< 3	6.67
		4.29	59		6.80

ON THE YIELD RATES IN THE INDIAN PRAWN PROCESSING INDUSTRY

by

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ABSTRACT

Data were collected from peeling sheds and processing factories in Cochin, Calicut, Mandapam, Vizagapatnam, Bhubaneswar, Paradeep, Puri and Calcutta. Peeling and deveining operations were done by the employees of these establishments under commercial conditions. Weighing was carried out by staff of CIFT. It was found that the rates of recovery of meat on peeling was independent of variations in the quantity of raw material taken. For regular collection of data whole prawns of uniform size were weighed and the weight and number of prawns were noted. Then the whole prawns were beheaded and the weight of the headless prawns recorded separately for each sample. The headless prawns were then peeled and the weight of meat recovered was noted for each sample. The yield rates, along with the 95% confidence intervals, were calculated from these date for all commercially important varieties of prawns.

INTRODUCTION

When prawns and fish are processed, the quantity of meat recovered is important information, useful for management and decision making in the processing industry. Unnithan *et al.* (1975), have worked out the average yield of different species of prawns on conversion from whole to peeled and deveined (PD) and Nair *et al.* (1979) have worked out the counts of whole prawns required for obtaining meat of specified size grades. The wastage due to spoilage in processing factories was as high as 35% (Nair *et al.* 1979) which was reflected in the average yield of prawns, at that time. A lot of research and development work has taken place during the past 15 to 20 years. The qualitative and quantitative improvement in raw material during this period would no doubt affect the yield rates of prawns. Therefore, a survey of the peeling sheds/factories was made in 1989 and 1990 to update and supplement the information on yield rates of commercially important prawns. This communication gives the results of the investigations.

MATERIALS AND METHODS

Method of collection of data

The yield of meat from 100, 200, 300, 400 and 500 g of whole prawns were recorded. Data were collected from peeling sheds and fish and prawn processing factories in Cochin, Kozhikode, Mandapam, Vizagapatnam, Bubaneswar, Puri, Paradeep and Calcutta. It was found that the rate of recovery of meat on peeling was independent of the variations in the quantity of raw material taken. For regular collection of data from the peeling sheds 200 to 500 g of whole prawns of uniform size were weighed and the weight and number of prawns were noted. The whole prawns from each sample were beheaded and the weight of headless prawns was recorded separately for each sample. Then the headless prawns were peeled and the weight of meat recovered was noted for each sample. Similar data were collected for two to three different samples of uniform size of material depending on the size range available on each day of collection. These data were collected separately for all species of prawns available on the day of collection. The origin of the raw material was noted and also the extent of spoilage. The peeling/deveining operations were done by the employees of the establishments. Weighing was done by staff of CIFT. To cross check the estimated yield rates with actual yield rates obtained, data on total quantity of whole prawns brought to the peeling sheds on a day and the total meat yielded from it were collected from some peeling sheds at Cochin.

Method of estimation of yield rates

From the number corresponding to each weight of the sample, the size grades were worked out. Size grades were calculated as number per pound of meat. The following size grades were observed: below 80, 80/120, 200/300, 300/500 and above 500.

The data were examined for working out a statistical relationship between quantity of prawn meat and quantity of raw material. For estimating the common ratio Y/X, three quantities $\Sigma XY/\Sigma X^2$, $\Sigma Y/\Sigma X$ and $\Sigma(Y/X)/n$ are all unbiased estimates of the population ratio, β (Snedecor and Cochran, 1968) (here, Y is the weight of prawn and X the weight of whole prawns or headless prawns). The estimate $b = \Sigma XY/\Sigma X^2$, appeared appropriate as the variance of $\in$ in the model $Y = \beta X + \in$ is constant (Snedecor and Cochran, 1968). Therefore this ratio was employed to work out the yield rates and the 95% confidence limits were worked out size-grade-wise and species-wise, separately for the east and west coasts of India.

RESULTS AND DISCUSSION

The quantity of whole prawns were plotted against the quantity of meat yielded from them of *M. dobsoni,* and *P. stylifera* (Fig. 1). The weight of whole prawns varied between 100 and 500 g. As the points lie close to a straight line, the rate of recovery of meat on peeling is independent of the variation in the quantity of raw material taken.

The yield in percent along with the 95% confidence intervals and the sample size for the commercially important varieties of prawns are presented in Tables 1 to 7. Wherever possible, yield rates of size grades have been worked out separately for east and west coasts of India. The salient features for the different varieties are discussed below.

(i) *M. dobsoni*: - A region-wise difference was observed in the meat recovered with a tendency for higher yield rates for the material from the east coast (59%) compared to the west coast (53%). The yield rate for the country as a whole worked out to 55 ± 1%. The variation in the yield rates for different size-grades did not show any pattern for either coast (Table 1).

The yield from whole to headless was higher for the east coast (73%) than that for the west coast (68%). This could perhaps be attributed to the difference in proportion of the weight of the head portion relative to the weight of whole prawns. The weight of the head portion appears to be higher for the west coast. The relatively lower yield rates of meat for the west coast also indicates this.

The yield rate from headless to meat was higher for the east coast (81%) than for the west coast (79%). Differences in the yield rates between size grades were not significant.

(ii) *P. stylifera*: - The recovery rates of meat from whole prawns were not significantly different for the two coasts for *P. stylifera*, the yield rate being 48% at the all-India level. A size grade-related yield was noted for this species, the larger size grades yielding more meat (Table 2). The trend was not so apparent for the east coast, perhaps because there was an insufficient number of samples in the smaller size grades (200/300 and 300/500). For the west coast sample size was also smaller for size-grade 300/500 as revealed by a confidence interval of 4% compared to 2 and 3% for the other size-grades.

On converting the whole prawns to headless prawns, the yield rate was found to be 64% for the east coast and 61% for the west coast. This difference is significant as judged from the confidence intervals. Size-grade-wise differences in the yield rates did not show any pattern (Table 2).

The yield rates from headless prawns to meat worked out to 78% for the country as a whole. Region-wise difference was not found to be significant, but differences in yield with size-grade appeared to be significant, larger sizes showing higher yield rates. For small sizes (300/500), the sample sizes were not large enough, as can be seen from the confidence interval of 3%.

(iii) *P. indicus*:- The yield rate of *P. indicus* from whole to meat worked out to 59 ± 2% for the west coast. Higher yield rates for larger size-grades 80/120 and below 80 were noted. There was insufficient data to make separate estimates for the east coast.

The yield rate was 70 ± 1.5%, when converted from whole to headless, for the west coast.

The yield rate on conversion from headless to meat was 84 ± 2% for the west coast. Data were not sufficient to make inferences for size-grades.

(iv) *M. affinis*:- For this species the yield rates were 57 ± 4% from whole to meat; 69 ± 4% from whole to headless and 83 ± 3% from headless to meat (Table 4). These estimates are for the west coast.

(v) *M. monoceros*:- The meat yielded from whole prawns was 57 ± 3%, the yield from whole to headless prawns was 70 ± 3% and the yield of meat from headless prawns was 82 ± 2%. The estimates are for the west coast (Table 5).

(vi) *P. monodon*:- The observed sizes of prawns of this variety were large, all the size-grades being below 35 per lb of meat. The yield rate from whole to headless was 67 ± 3.5% and from headless to meat, 82 ± 3%. These estimates are for the east coast (Table 6).

(vii) *P. semisulcatus*:- As the observed prawns were landed in the headless condition, only the yield of meat from headless prawns could be determined. This was 81 ± 3% and the estimate is for the east coast (Table 7).

In general, large sized prawns are packed as headless and medium and small ones as meat. To reduce spoilage, the practice of removing the heads as soon as the prawns are caught, also prevails, especially when fishing is done off-shore. Therefore yield rates could not be worked out for whole to meat, whole to headless and headless to meat for some species.

For easy comparison among species, the yield rates are presented graphically in Figure 2. The bar diagram shows more or less the same pattern of variation in the three types of yield rates for all the species.

As a cross check the estimated yield on the total quantity of whole prawns brought to the peeling sheds on a day and the actual total meat yields were collected from some peeling sheds for the species *M. donsoni* and *P. sytlifera*. The actual yield rates worked out from these agreed very well with the estimated yield rates.

CONCLUSION

The rate of recovery of meat on peeling is independent of variation in the quantity of raw material taken. Though influence of region and size grade on yield was not found in all the species, these differences could be established in two species, *M. dobsoni* and *P. stylifera*. A regional difference in the yield was observed for *M. dobsoni* and size grade differences in *P. stylifera*, where larger size-grades yielded more meat.

The yield rates in percent for the different species can be summarized as follows:

Species	Whole to headless	Whole to meat	Headless to meat
M. dobsoni	69 ± 1	55 ± 1	80 ± 1
P. stylifera	62 ± 1	48 ± 1	78 ± 1
P. indicus	70 ± 1	60 ± 2	84 ± 1.5
M. affinis	69 ± 4	57 ± 4	83 ± 2
P. monoceros	70 ± 3	57 ± 3	82 ± 2
P. semisulcatus	-	-	84 ± 3
P. monodon	67 ± 3.5	-	-

ACKNOWLEDGEMENT

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Table 1: Region-wise and size-grade-wise yield (Sample size in brackets) *M. dobsoni* (Whole to meat) yield rate (in percent)

Size grade	East coast	West coast	All India
Below 80	57.6 (1)	55.5 ± 9 (2)	56 ± 3 (3)
80/120	56 ± 16 (3)	54 ± 2 (14)	54 ± 2 (17)
120/200	59 ± 3.5 (11)	54 ± 3 (11)	57 ± 2.5 (22)
200/300	59 ± 3 (7)	51 ± 2.5 (9)	54.5 ± 3 (I.5)
300/500	57 ± 7 (1)	54 ± 2.5 (11)	54 ± 2.5 (12)
Above 500	No sample (6)	55 ± 3 (6)	55 ± 3
Regardless of size-grade	59 ± 2 (24)	53 ± 1 (51)	55 ± 1 (75)

M. dobsoni (whole to HL)

Size grade	East coast	West coast	All India
Below 80	69 (1)	67 ± 37 (2)	67 ± 9 (3)
80/120	74 (1)	68 ± 3 (14)	68.5 ± 2 (17)
120/200	72 ± 3 (11)	69 ± 3 (11)	70 ± 2 (22)
200/300	75 ± 5 (7)	66 ± 2.5 (9)	70 ± 3 (I6)
300/500	69 (1)	69 ± 2 (11)	69 ± 2 (12)
Above 500	No sample	68 ± 5 (6)	-
Regardless of size-grade	73 ± 3 (23)	68 ± 1 (54)	69 ± 1 (77)

M. dobsoni (HL to meat) yield rate in percent

Size grade	East coast	West coast	All India
Below 80	81 ± 19 (2)	79.4 ± 8 (5)	80 ± 5 (7)
80/120	79.5 ± 11 (4)	80 ± 2 (14)	80 ± 2 (18)
120/200	83 ± 3 (12)	79 ± 2.5 (12)	81 ± 2 (24)
200/300	79.5 ± 2.5 (7)	77 ± 5 (9)	78 ± 3 (16)
300/500	83 (1)	78 ± 3.5 (11)	78 ± 3 (12)
Above 500	no sample	81 ± 6 (6)	-
Regardless of size-grade	81 ± 2 (26)	79 ± 1 (57)	80 ± 1 (83)

Table 2: *P. stylifera* (Whole to meat) yield rate (in percent)

Size grade	East coast	West coast	All India
80/120	50. ± 5 (4) (9)	51 ± 3 (13)	51 ± 2
120/200	49 ± 3 (7) (22)	48 ± 2 (29)	48 ± 1.5
200/300	47 (7) (9)	46.5 ± 2 (16)	46.5 ± 2
300/500	57 (2) (10)	47 ± 4 (12)	47 ± 3
Regardless of size-grade	49 ± 2 (15)	48 ± 1 (65)	48 ± 1 (80)

Size grade	East coast	West coast	All India
80/120	63 ± 4.5 (4) (9)	63 ± 4 (13)	63 ± 2.5
120/200	63 ± 3 (7) (22)	60 ± 2 (29)	60 ± 1.5
200/300	67 (1) (23)	61 ± 2 (24)	61 ± 2
300/500	74 (2) (11)	62 ± 3 (13)	62 ± 3
Regardless of size-grade	64 ± 2 (15)	61 ± 1 (65)	62 ± 1 (80)

P. stylifera (HL to meat)

Size grade	East coast	West coast	All India
80/120	80. ± 11 (4) (9)	82 ± 1 (13)	81 ± 2
120/200	77 ± 5 (7) (21)	81 ± 2 (29)	80 ± 2
200/300	70 (1) (23)	76 ± 2 (24)	76 ± 2
300/500	77 (2) (11)	76 ± 3 (13)	76 ± 3
Regardless of size-grade	76.5 ± 3.5 (15)	78 ± 1 (65)	78 ± 1 (80)

Table 3. *Penaeus indicus* (whole to meat)

Size grade	East coast	West coast	All India
Below 80	62 (2) (12)	60.5 ± 3 (14)	61 ± 3
80/120	65 (2) (9)	61 ± 6 (11)	62 ± 4
120/200	- (1) (12)	57 ± 4	-
200/300	61 ± 5 (6) (2)	59	-
Regardless of size-grade	61 ± 5 (6) (35)	59 ± 2 (41)	60 ± 2
Whole to HL			
Below 80	70 (2) (11)	72 ± 2 (13)	71 ± 2
80/120	77 (2) (9)	70 ± 4 (11)	71 ± 4
120/200	- (1) (12)	69 ± 4	-
200/300	70 ± 4 (8) (41)	70 ± 1.5 (49)	70 ± 1
Regardless of size-grade	70 ± 4 (8) (41)	70 ± 1.5 (49)	70 ± 1
HL to meat			
Below 80	83. ± 4 (6) (11)	84 ± 4 (17)	84 ± 3
80/120	82 ± 3 (5) (9)	87 ± 2.5 (14)	85 ± 2
120/200	80 ± 3 (4) (12)	83 ± 2.5 (16)	82 ± 2
200/300	- (1) (2)	79 ± 3	-
Regardless of size-grade	82 ± 3 (10)	84 ± 2 (34)	84 ± 1.5 (44)

Table 4. *M. affinis* (whole to meat)

Size grade	East Coast	West Coast
Below 80	-	57.5 ± 5
80/120	-	54 ± 11
Regardless of size-grade	*58 ± 2 (4)	57 ± 4 (14)
Whole to HL		
Below 80	-	70 ± 4 (11)
80/120	-	64 ± 15 (3)
Regardless of size-grade	74 ± 4 (4)	69 ± 4 (14)
HL to meat		
Below 80	-	82 ± 3 (12)
80/120	-	85 ± 16 (3)
Regardless of size-grade	79 ± 3 (4)	83 ± 3 (15)

*Pertaining to Chilka Lake

Table 5. *M. Monoceros* (whole to meat)

Size grade	East Coast	West Coast
80/120	-	63. ± 9 (4)
120/200	-	57.5 ± 3 (10)
200/300	-	50 ± 9 (5)
300/500	-	52 (2)
Regardless of size-grade	-	57 ± 3 (21)
Whole to HL		
80/120	-	73. ± 5 (4)
120/200	-	71 ± 4 (10)
200/300	-	64 ± 9 (5)
300/500	-	65 (2)
Regardless of size-grade	-	70 ± 3 (21)
HL to meat		
80/120	-	86 ± 7 (4)
120/200	-	81 ± 3 (10)
200/300	-	79 ± 4.5 (5)
300/500	-	80 (2)
Regardless of size-grade	-	82 ± 2 (21)

Table 6 *P. monodon* (whole to HL)

Size grade	East Coast	West Coast
26/30 and under	66.6 ± 4 (3)	-
31/35 and above	-	-
Regardless of size grade	67 ± 3.5 (4)	-
HL to meat		
26/30 and under	84 ± 12 (4)	-
31/35 and above	81 ± 2 (5)	-
Regardless of size grade	82 ± 3 (9)	-

Table 7 *P. semisulcatus* (Heatless to meat)

Size grade	East Coast
Below 80	81 ± 3 (8)
80/120 and above	82.5 ± 7 (4)
Regardless of size grade	81 ± 2 (12)

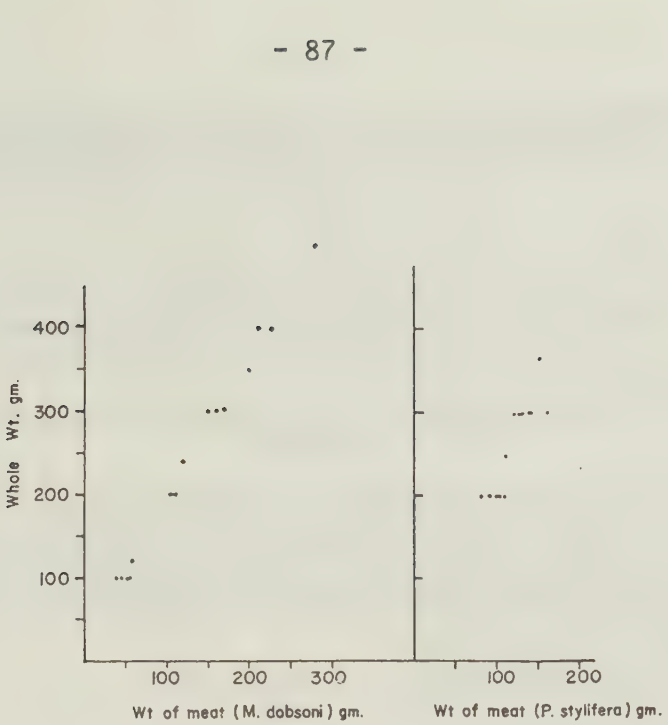

Fig. I. Plot of meat recovered against the quantity of whole prawns taken.

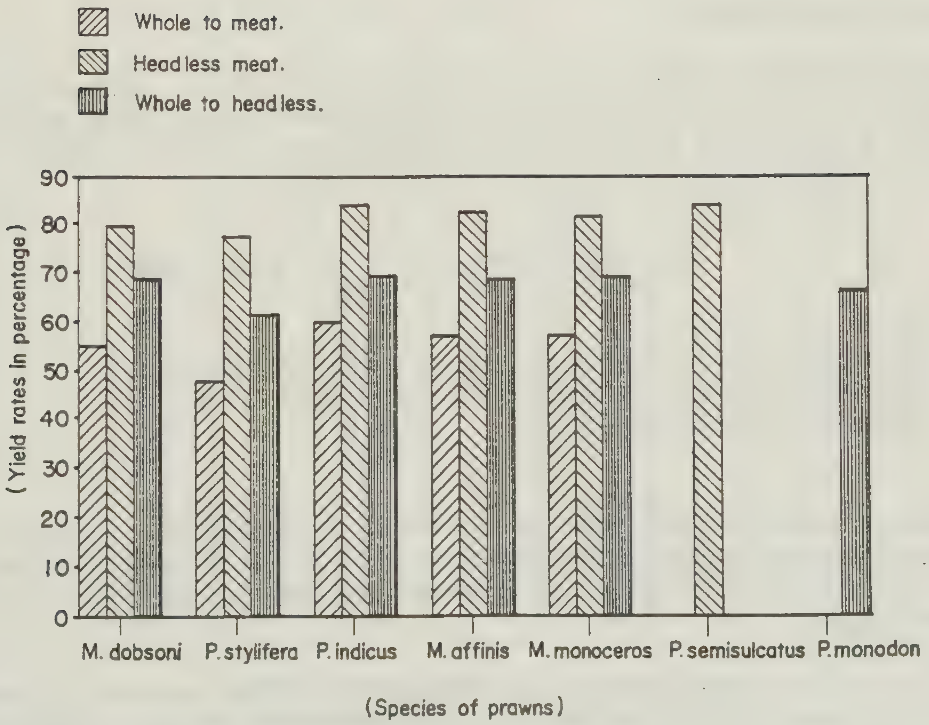

FIG. 2. YIELD RATES IN PERCENT

CORRELATION OF ORGANOLEPTIC QUALITY TO CAROTENOID CONTENT AND COLOUR OF CULTURED SHRIMP *(Penaeus Monodon)*

by

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ABSTRACT

Sensory tests on colour correlated to carotenoid content of pond shrimps (*Penaeus monodon*) have been conducted. Astaxanthin is the major carotenoid compound in pond shrimps. The level of astaxanthin influences the colour of shrimps which results in producing different intensities of pink colour during cooking.

INTRODUCTION

In the development of pond shrimp culture differences in shrimp colours, especially of the intensivly cultured pond shrimp, have been encountered. Several buyers, especially Japanese, set different prices for black pond shrimps from shrimp of other colours, blue in particular. The differences range up to 10% higher for black pond shrimp even though the sizes and qualities of the black and of other colours are the same.

This paper presents variation of organoleptic quality as well as colour differences through observation.

MATERIALS AND METHOD

The samples were taken from the cold storage of Mina Utama Ltd., Sidoarjo, East Java, in the months of January and February 1989. The observation on shrimp colour percentages was based on random sampling of the arriving shrimps at the processing unit, where the colour observation was conducted by calculating the weight percentages through the formula:

$$\boxed{A = X/b \times 100\%}$$ in which:

A = The colour percentage of the shrimps received at the cold storage.
X = The weight of the shrimps in the sample having the same colour.
b = The weight of the samples taken.

To identify the organoleptic differences between the shrimp colour, the shrimps were grouped according to their colours and the toughness of the shell was examined by the panellists of the National Centre for Fish Quality and Processing Technology Development (NCQC) after the shrimps were thawed.

The observation on organoleptic quality differences was also conducted by steaming the shrimps for 10 minutes (at 100°C) and also by frying them for three minutes in boiling cooking oil. The panellists of NCQC then examined the differences in tastes and colours.

Qualitative tests on pigment were conducted by extracting pigments from each type of shrimp colour. The extraction method was that of Bligh and Dyer which was then diluted 10 x with chloroform. Scanning was then conducted between 400 - 700 n.m. with a Spectronic 20, after which the maximum absorption was evaluated.

RESULT AND DISCUSSION

The colour percentage of shrimps arriving at the cold storage

In the examination of colour of the shrimps arriving at the factory during a ten day period ten types of colours and composition were documented. The colour types and composition of the observed pond shrimps are listed in Table 1.

Table 1: Percentage distribution of different colour for cultured shrimp for intensive and traditional pond

Brackish water pond	Percentage (1%)									
	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
Intensive	-	-	-	-	-	38,2	17,6	15,8	25,8	3,6
Intensive	-	-	-	-	-	16,0	24,4	42,7	13,5	3,5
Traditional	-	25,6	52,7	21,7	-	-	-	-	-	-
Intensive	-	-	-	-	-	29,0	18,9	26,7	24,5	0,9
Traditional	30,2	41,7	28,1	-	-	-	-	-	-	-
Intensive	-	-	-	-	-	12,9	27,7	25,8	32,7	3,7
Intensive	-	-	-	-	-	10,1	26,6	28,9	29,8	4,7
Traditional	-	-	43,4	23,8	32,4	-	-	-	-	-
Intensive	-	-	-	-	-	8,5	33,6	19,6	36,7	1,5
Traditional	12,5	-	5,8	-	-	-	-	-	-	-
Intensive	-	-	-	-	-	10,1	23,8	44,5	4,6	18.0
Traditional	21,7	41,1	37,5	-	-	-	-	-	-	-
Intensive	-	3	-	-	-	3,1	45,2	25,2	24,6	1,8
Intensive	-	-	-	-	-	1,2	21,7	31,6	36,7	2,6
Traditional	33,9	-	-	69,9	-	-	-	-	-	-
Intensive	-	-	-	-	-	12,6	31,6	18,4	37,4	-
		-								

A1: brownish black
A2: blackish green
A3: blackish brown
A4: whitish green
A5: milky brown
B1: rather dark brown
B2: whitish brown
B3: brown rather blueish
B4: light blue
B5: blue

This descriptive information is obtained in accordance with that given by the company.

As shown in Table 2, shrimps coming from traditional ponds are mostly classified in A colours, which range from brownish black to milky brown, with no blue element at all, while the shrimps raised at intensive ponds show the blue element, especially for B3, B4 and B5. These colour differences seem to fit a number of reports on colour differences of pond shrimps issued by some observers. As to causes of colour differences, there is uncertainty with geographical location, environmental factors as well as sanitation and hygiene being possibilities. Further investigations are required.

Pond shrimp pigments in several types of colours

The shrimp colour was extracted by the method of Bligh and Dyer to avoid pigment degradation as the method does not involve any heating.

Scanning from photometric test results show that the ten colours of shrimps show a similar pattern with maximum absorption at 475 nm as indicated in Figure 1.

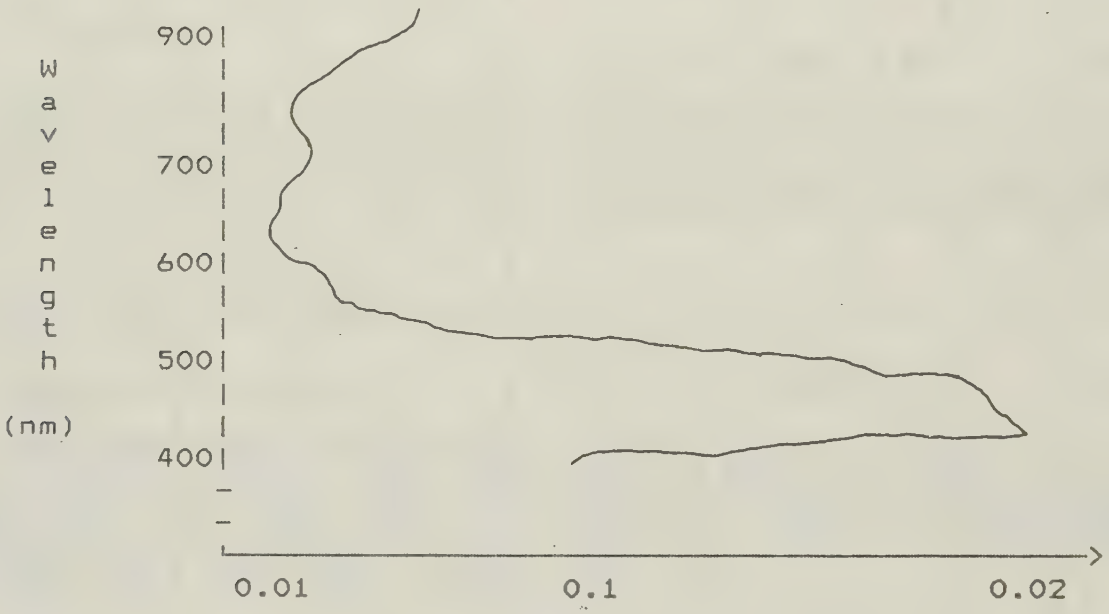

Figure 1. Scanning of absorption of pigment from cultured shrimp (from 400 nm to 800 nm)

Chen and Mayer (1984) found that astaxanthin was the dominant pigment in shrimps, with maximum absorption at 475 nm; 493 nm and 513 nm (Anon, 1967). The scanning analyses shows only one peak of maximum absorption at 475 nm. This may have been because the Spectronic 20, using a galvanometer, is unable to detect the small absorption difference between 475, 493 and 513 nm. However, the ten colour types have similar absorption scanning patterns indicating that the dominant pigment in those 10 colour types is astaxanthin with a highest peak of maximum absorption at 475 nm.

From the qualitative point of view, it can be seen that the height of the peak at 475 nm is different for each colour type as shown in Table 2.

Table 2: Absorbance (at 475 nm) of carotenoid content for 10 colour types of shrimp

Colour types of shrimp	Absorbance
A1	0.324
A2	0.389
A3	0.562
A4	0.216
A5	0.280
B1	0.274
B2	0.174
B3	0.090
B4	0.069
B5	0.040

Table 2 shows clearly that the height of the peak of maximum absorption of shrimp from group B correlates with the colour of shrimp shell, the darker colour (blackish) has higher absorption than the bluer one. This indicates that the higher concentration of pigment results in darker colour. Although the maximum absorption data for shrimps of group A is relatively less favourable, there seems to be a correlation between the shell colour and pigment (astaxanthin) content.

Organoleptic quality of various types of pond shrimp colours

The panelists found it difficult to differentiate between the various colours with respect to shell toughness, both before and after freezing. However, it was possible to distinguish differences between groups A, traditional pond shrimp, and group B, intensively cultivated shrimp which were softer. It is important to determine the causes as it will affect the quality of export shrimp which are likely to be mixed in frozen stocks.

Table 3 The levels of the colour brightness for ten colour types of shrimp after cooking

Colour types of shrimp	Levels of the brightness
A1	1
A2	2
A3	3 Brighter
A4	4
A5	5 ^
B1	1
B2	2
B3	8
B4	9
B5	10

Table 3 shows clearly that the black shrimp have a higher brightness compared to the blue ones. The change of shrimp colour after cooking is caused by the heating process which results in oxidation pf the pigment. The shrimp pigment astaxanthin (3.3 dihidroxy - 4.4 diketone) carotenoid will be coverted to astasin when oxidized by heating.

Although the panelists did not differentiate between the taste of shrimps of the different colours, it seems that the colour of shrimp after cooking might be the reason that the blue shrimp are priced 10% lower than the black ones. This can be easily understood because the consumer will have a higher appreciation of a better colour in the final cooked product.

CONCLUSIONS AND SUGGESTIONS

From the test results, it can be concluded that:

1. The percentage of blue shrimp in intensive ponds is higher.

2. The pigment of all types of shrimp colour has a similar peak of maximum absorption at 475 nm. This is possibly astaxanthin, and at low levels it causes a bluish colour of shrimp.

3. The buyers set different prices due to the differences of colour after the shrimps are heated.

Considering the above conclusions, it is advisable to further investigate the correlation between pond shrimp colour and astaxanthin. If shrimps colours are affected by astaxanthin, feeding trials are required to find out whether the colour of pond shrimp can be improved by adding astaxanthin to shrimp feed. When astaxanthin improves colour, the possibility of using shrimp head or shell meal as an astaxanthin substitute should be considered.

ACKNOWLEDGEMENTS

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DETERMINATION OF FILTH AND MICROBIOLOGICAL QUALITY OF SHRIMP PASTE IN THAILAND

by

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ABSTRACT

Shrimp paste has been used in Thailand and some Southeast Asian countries for a long time. Since filth and microbiological quality is the sign of improper manufacturing practice, 36 shrimp paste samples from different parts of Thailand were analyzed. The average value of a_w and salt were 0.70 and 22% respectively. The method of light filth analysis for shrimp paste was modified from AOAC (1984) #44.069. The filth commonly found in shrimp past was ants and human hair. Other foreign matter found were small pieces of water plant, pieces of newspaper and other kinds of crustacea. Microbiological analysis of shrimp paste showed that there were no *Salmonella*, faecal coliforms or *E. Coli* an any sample. Coagulase negative *Staphylococcus aureus* was found in some low price samples with the average value of 8 x 10 colony/gm. *Cl. perfringens* was found in more than 50% of the samples with numbers ranging from 1.5×10^2 - 1.0×10^4 colony/gm. For wholesomeness it is suggested that thorough heating is required when shrimp paste is used for cooking. The highest mold count found in a low price sample was 2.6×10^3 colony/gm. Total aerobic plate count detected by using trypticase soy agar (TSA) with the addition of 5% NaC1 showed values of 2.5×10^3 - 7.3×10^3 colony/gm.

Analysis of results according to selling price indicated that higher priced samples were better in quality. This implies better production GMP.

INTRODUCTION

Shrimp paste is popular in Thai food and some other Southeast Asian countries for a long time. The shrimp paste industry in Thailand is a traditional industry which needs to upgrade its technology, production methods and techniques. The majority of the products are consumed locally and some are exported. As a guideline for quality improvement, the Thai Industrial Standards Institute is now in the process of setting up a standard for shrimp paste. At the same time, this project was undertaken to determine the hygienic quality of shrimp paste in order to indicate points for quality improvement and further process development.

METHODS

Sampling plan

Shrimp paste samples were bought from the local markets of six different provinces: Samutprakarn, Samutsakorn, Samutsongkram, Junthaburee, Trad and Ranong. These provinces are close to the sea, therefore production of shrimp paste can be found. Shrimp paste samples were classified into three groups according to selling price as listed in Table 1. Since shrimp paste production is a family or small-scale industry, two different shrimp paste producers were sampled. The complete sampling plan of this experiment is shown in Figure 1.

Analysis of shrimp paste samples

Analysis of shrimp paste samples were set up to determine some chemical properties, microbiological properties and filth and foreign matter content.

Chemical analysis

i. Water activity (a_w) (Novasina thermo constanter model THE-2/BS J
ii. % sodium chloride (NaCl) (AOAC, 1984 # 34.005)
iii. pH and %acid insoluble ash (AOAC, 1984 # 31.012)

Microbiological analysis

Each shrimp paste sample was analyzed for total aerobic plate count using tripticase soy agar plus 5% NaCl incubated at 32°C for 2-4 days. The methods of analysis as stated in APHA (1984) were followed. Also, mold count of the sample was determined by using dichloran 18% glycerol agar. The analysis of food poisoning bacteria in shrimp paste was also undertaken as follows: detection of coagulase positive *S. aureus* by surface plating (APHA, 1984) faecal coliform bacteria and *Salmonella* determination by APHA (1984) and *Cl. perfringens* detection by the method of Shahidi (1971).

Light filth analysis

Duplicate samples were used for light filth determination. The method of filth analysis from AOAC (1984) # 44.069 was followed and modified by using 10-25 gms shrimp paste sample and hydrochloric acid (30 ml - 12N HCl in 970 ml distilled water). 600 ml was mixed with the sample right before the flotation step (Akepanidtavorn, 1991).

RESULTS AND DISCUSSION

The chemical properties of shrimp paste determined from 36 samples had average values as follows: a_w 0.7, pH 6.82, %NaCl 22 and % acid insoluble ash 1.18 (dry basis) (Table 2). The a_w value of shrimp paste varied within selling price groups and the area of production. The highest a_w value was 0.77 which obtained from high price shrimp paste from Samutsongkram where traditional shrimp paste production favours a rather wet product. According to Rodel (1975) and Leistner and Rodel (1976a) (as cited by Vibulsresth, 1984), meat products with a_w 0.91 can be stored with no refrigeration required. Growth of most pathogenic bacteria in food can be inhibited by water activity levels of less than 0.85 (Chirife, 1989. However, it is necessary to lower a_w value to 0.7-0.75 in order to stop yeast and mold growth (Chirife, 1989). The a_w of all shrimp paste samples in this experiment could lead to the conclusion that there should be no pathogenic bacterial growth in traditional shrimp paste from Thailand. The pH value of shrimp paste ranged from 6.07 to 7.68 which are normally characteristic of salted cured meat products. It is suspected that the low pH value of 5.22 resulted from the addition of a carbohydrate source to shrimp paste. The low price product tends to have a high salt content. Acid insoluble ash in the products also varied considerably with the value range from 0.38 to 2.57%. This value is related to the amount of sand and soil particles in shrimp paste. During the cleaning process of raw materials if the flotation method is used it would help separating most of the heavy filth. Also, the producer should be aware of contamination with sand and soil particles during process.

Microbiological quality of shrimp paste

The results showed that there were no *Salmonella*, faecal coliforms or *S. aureus* coagulase positives in shrimp paste. *Cl. perfringens* can be detected in most of the shrimp paste samples with the value around 10^3 colonies/gm. According to Duncan (1980), consumption of vegetative cells of *Cl. perfringens* up to 10^6 colonies/gm will cause food poisoning symptoms. Although the high salt content and low a_w value of shrimp paste can inhibit growth of this bacteria, bacterial spores will still survive. Therefore, sanitation processes are required in order to prevent contamination with this bacteria.

The same practices are required to reduce mold and total plate count in shrimp paste.

Filth determination of shrimp paste

Foreign matter found in shrimp paste included small crabs, unidentified zooplankton, unidentified insect fragments, pieces of plastic film, nylon string and newspaper, ants, human hair, and pieces of water plant. The most objectionable matter resulting from insanitary conditions was defined for defect score and notation according to Table 4. The low-priced product showed the highest defect scores (Table 5) since sanitary control required greater production cost. Other foreign matter such as small crabs, pieces of newspaper, water plant and nylon string can be separated by sorting and cleaning processes. Some simple devices may be introduced to upgrade these processes.

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Table 1 Price range of shrimp paste bought from local market in six provinces

Provinces	Selling price (Bhat/kg)		
	Low	Moderate	High
Samutprakarn	25-30	40	50
Samutsakorn	25	40-50	60-80
Samutsongkram	25-30	40	50
Junthaburee	30-40	50-60	70-80
Trad	25-30	50-60	70-80
Ranong	20-25	30-35	40-50

Table 2 Chemical properties of shrimp paste which were sampled by selling price and area of production

Chem. prop.	Price class	Area of Production						
		Samut-songkram	Samut-prakarn	Samut-sakorn	Ranong	Juthaburee	Trad	Average
A_w	Low A	0.72	0.69	0.71	0.67	0.71	0.73	0.70
	Low B	0.72	0.70	0.69	0.69	0.67	0.67	
	Moderate C	0.75	0.72	0.71	0.67	0.70	0.70	0.71
	Moderate D	0.76	0.71	0.73	0.67	0.71	0.68	
	High E	0.77	0.67	0.66	0.68	0.70	0.66	0.69
	High F	0.77	0.68	0.70	0.67	0.66	0.66	
	Average	0.75	0.70	0.70	0.68	0.69	0.68	0.70
% NaCl	Low A	22.04	34.80	19.72	22.23	25.52	21.46	25.04
	Low B	40.02	19.42	27.84	17.40	22.62	27.26	
	Moderate C	16.24	24.94	24.94	19.43	22.04	19.72	21.19
	Moderate D	20.30	29.00	20.30	19.14	17.40	20.88	
	High E	19.14	24.36	20.30	14.79	18.56	19.72	20.21
	High F	18.56	20.01	22.04	23.78	20.30	20.88	
	Average	22.72	25.42	19.48	21.07	21.73	21.73	22.14

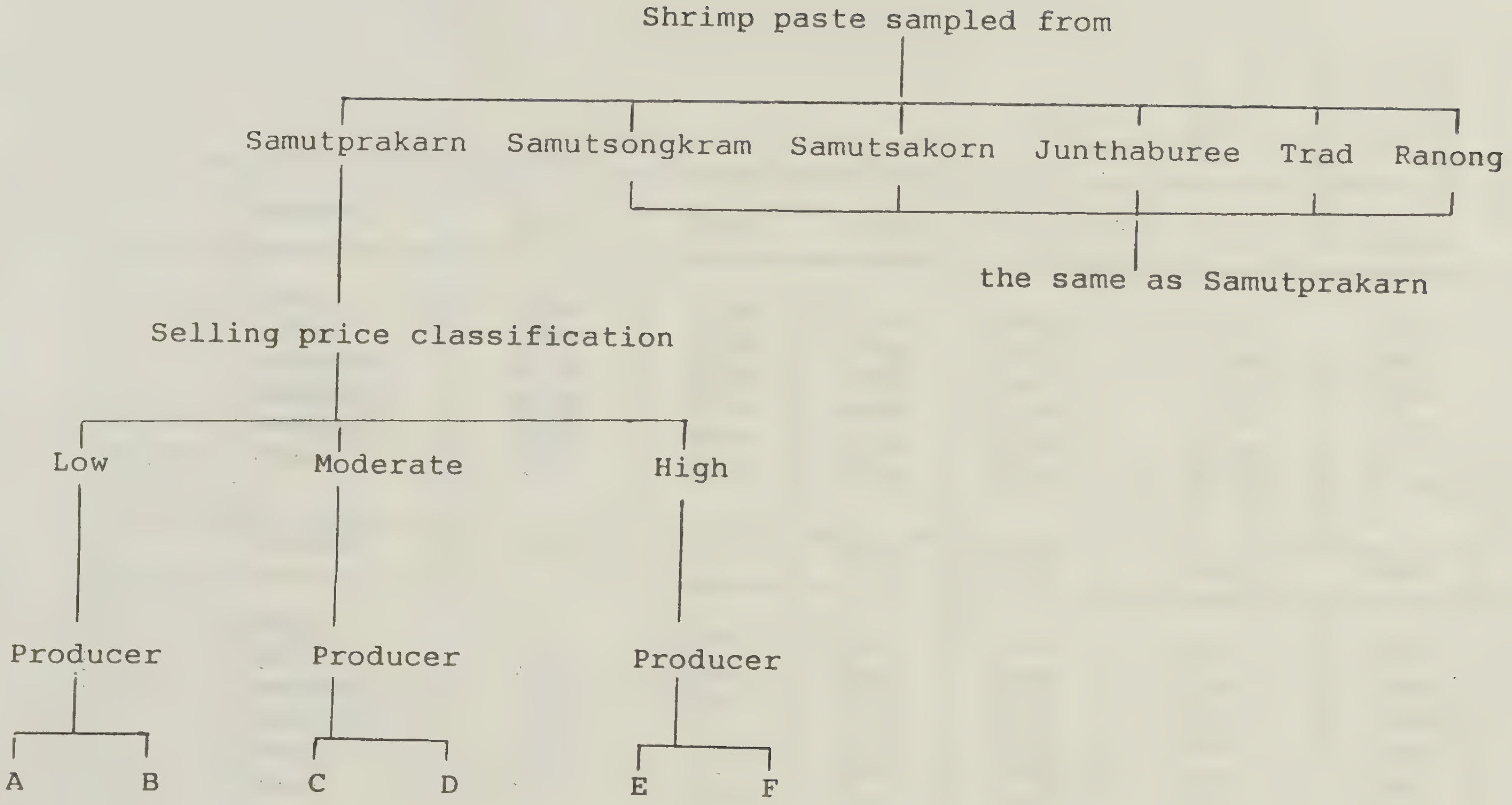
Shrimp paste sampled from
Samutprakarn
Samutsongkram
Samutsakorn
Junthaburee
Trad
Ranong
the same as Samutprakarn
Selling price classification
Low
Moderate
High
Producer
Producer
Producer
A
B
C
D
E
F

Figure 1 Shows the sampling plan of shrimp paste observed in this experiment

IMPACT OF BRACKISHWATER SHRIMP CULTURE ON THE INTERNATIONAL SHRIMP MARKET

by

Sumpeno Putro

ABSTRACT

Today, shrimp is the most important high value seafood product in international markets with an excellent potential as an export item and foreign exchange source, especially for countries with the requisite resources. It is anticipated that the demand for shrimp will continue to grow in the three principal markets - the United States of America, Japan and Western Europe. Although many people still believe that the oceans contain an enormous untapped supply of marine fishery resources, including shrimp, catch trends for the world's principal shrimp resources indicate that they are at or close to their Maximum Sustainable Yield (MSY), or in some areas the stocks have been overfished. Additional supplies, therefore, have recently and will continue to rely on aquaculture.

In recent years, many developing countries and international development institutions have invested substantial resources in brackishwater shrimp aquaculture on the assumption that the so-called "Blue Revolution" will generate hard currency earnings, cheap protein and more employment for rural people. However, heavy arrivals of black tiger shrimp from new producing countries in South East Asia drove prices down by 40-50% in the second half of 1989.

Although it is unlikely that shrimp prices will return to what they used to be, it is apparent that the tremendous production increase from brackishwater shrimp culture has prompted many producing countries to be more cautious of a further slide in prices, which could cause a disaster to the industry. In addition, various technical problems also need immediate attention in order to sustain brackishwater shrimp culture production, especially unreasonably high stocking density, deterioration of grow-out ponds, poor choice or artificial feed, as well as pathogenic factors.

Indiscriminate use of medicine and antibiotics coupled with inadvertent use of polluted water could severely damage the quality of cultured prawn and further hinder a greater role in the national economy.

INTRODUCTION

Indonesia's seafood exports soared by 50 percent in value in 1988 to reach a new record total estimated at US$714 million. They rose 29 percent in volume to 181,000 metric tons.

Shrimp was, by a long way, the biggest contributor in this export surge, with earnings of around $500 million. Shrimp export during 1989 and 1990 showed further increase with rising inputs from the shrimp farming industry. It is anticipated that farmed shrimp will continue to play a greater role in the world's shrimp industry in the coming days.

TRENDS IN BRACKISHWATER SHRIMP CULTURE

Traditional extensive brackishwater shrimp culture has been practised in many countries since time immemorial. In Asia brackishwater shrimp are commonly raised in mixed culture with milkfish (*Chanos chanos*). However, in recent years the technology for production of brackishwater shrimp, especially the black tiger *Penaeus monodon* has moved towards an intensive export oriented aquaculture which has become a billion dollar business.

The brackishwater shrimp production has increased dramatically since then with concomitant increment of culture areas, as shown in Figure 1. The world shrimp landings increased from 1,864 million MT in 1984 to 2,038 million MT in 1987 with an average annual growth rate of 9.0% In contrast, the growth rates of brackishwater shrimp culture were 25% in 1987 and 27% in 1988, respectively.

Asian countries played an important role in this accelerated growth. Of the estimated production of 560,000 MT of cultured shrimp in the world in 1988, 85.2% were produced by Asian countries. The major contributor to this so-called "Blue Revolution" is China, followed by Indonesia, Thailand, Taiwan (Province of China), the Philippines, India, Vietnam and Bangladesh, as shown in Table 1.

INDONESIAN BRACKISHWATER SHRIMP CULTURE INDUSTRY

The Indonesian shrimp industry has expanded to the point of being a world production leader. Exports have increased from 26,166 metric tons in 1989, representing the generation of more than US$788,760,000 in export earnings. Shrimp production ponds are concentrated in five areas; South Sulawesi, East Java, Central Java, West Java, Aceh and North Sumatra. Additional opportunities for development are possible in the relatively remote provinces of East and South Kalimantan, Maluku, and Irian Jaya. As the shrimp industry has grown, land use conflicts have developed particularly with respect to rice production, tourism and coastal and mangrove ecology.

Three levels of management are used to produce shrimp: extensive, semi-extensive and intensive. Extensive systems tend to be stocked at low densities, use low-input levels, employ fewer labourers per hectare, produce high quality shrimp and operate at less risk. Intensive systems are stocked at high densities (over 30 PLs per square metre), are capital intensive, tend to utilize all available technologies, and operate at greater financial risk. Water quality deterioration and lack of technical ability are usually limiting factors to shrimp farm intensification. Shrimp react to deteriorating water quality by succumbing to largely opportunistic disease. Average production parameters for an intensive operation are given in Table 1. Table 2 displays an economic comparison between extensive, semi-intensive and intensive production systems.

Table 1 Production costs per cycle (2 cycles per year) for Indonesia shrimp ponds managed intensively (adapted from Chamberlain, 1990)

Production Parameters	
Stocking Density (#/sq.m)	30
Feed Cost/kg (RP.)	2,200
Food Conversation Rate (%)	1.8
Survival Rate (%)	60
Length of Cycle (months)	4
Size at Harvest (#/kg)	30
Production (kg/ha)	5,625
Price per kg (Rp.)	10,000
Capital Cost	**RP/ha**
Land Cost	15,000
Pond Construction	8,000
Electrical Generator and Distribution	8,000
Aerators, Pump and Equipment	15,000
Miscellaneous (buildings, fencing, lighting, etc.)	4,000
Total	50,000
Operating Cost	**RP (million)**
Penaeus monodon fry (Rp. 12 each)	3.60
Feed	22.28
Labour (4 months @ RP 300,000/ha/mo)	1.20
Electricity (4 months @ RP. 225,000/ha/mo)	0.90
Miscellaneous (transportation, repairs, chemicals, etc.)	2.00
Interest (10% of capital costs)	5.00
Total	34.98
Revenue	**RP (million)**
Gross, 5625 kg/ha @ Rp. 12,500/kg	70.31
Net, gross minus operating	49.04
Breakeven Price	
Operating Cost/Production (RP/kg)	6,219

Table 2. Economic comparison of extensive, semi-intensive and intensive shrimp farms (adapted from Chamberlain, 1990)

	Management System		
Parameter	Extensive	Semi-intensive	Intensive
Land Purchase	Limited	Limited	Yes
Capital cost (Rp/ha)	Low	6-7 million	50 million
Production (kg/ha/cycle)	179	1000	5625
Operating Cost (million Rp/ha/cycle)	0.6	4.98	35
Breakeven Cost (Rp/kg/cycle)	3352	4980	6219

Husbandry practices and disease management

The primary constraint to successful shrimp farming in Indonesia is poor quality management as evidenced by increasing disease occurrence. Infectious diseases and other health problems are often the result of environmental disfunction and can be moderated by good management. While dependence on chemotherapeutics varies among farm managers, many managers with access to adequate water resources use fewer drugs and have fewer disease outbreaks.

Site selection also has a great impact on disease occurrence. Location-induced water quality problems include acid soils and high turbidity in Sumatra, low salinity during rainy seasons in upstream ponds, and heavy metal contamination in Kendari, Sulawesi. Improved positioning of water intakes would measurably increase water quality in many farms by decreasing organic loading and by preventing the movement of potentially disease-laden waste one farm into another.

Feeds

Feed consumption by the shrimp industry in 1989 was estimated to be 100,000 MT. Feed demand is expected to grow about 30% annually to a 1994 consumption of 480,000 MT. Seventy percent of the shrimp feeds consumed in Indonesia is imported under 19 labels (primarily from Taiwan Province, but also mainland China and Germany). Fourteen domestic companies also produced feeds. In order to adjust to the changing nutritional requirements of growing shrimp, 2-3 different types of feed are available from most suppliers.

Most available feeds are expensive, high quality diets targeted for intensive systems. Intensive feeds exceed the needs of extensive and semi-intensive farms providing no supplemental fresh feeds. The cost of feed per kg of shrimp is approximately Rp. 3,750, which represents 60 percent of total production cost in a typical intensive farm.

Seedstock production

In 1987, 81 hatcheries were in operation in Indonesia but by 1990 this number had increased to an estimated 200. Environmental problems impacting one hatchery would be likely tp affect those in close proximity.

Hatcheries in Indonesia can be grouped into three categories: commercial, government and backyard. Commercial hatcheries are independent fry production units that generally include systems for water treatment, broodstock maturation and spawning, hatching of eggs, phytoplankton culture, larval culture and larval packing and transport. Most hatchery sites are affected by deteriorating water quality during the rainy season due to the influence of nearby rivers. During the rainy season, salinities fall, temperatures drop and levels of suspended solids and bacteria increase considerably.

Although broodstock quantities and prices vary seasonally, broodstock are rarely unavailable. Price for females typically ranges from a low of Rp 11,000 for tambak-raised broodstock to Rp. 15,000 - 50,000 (depending on shipping distance and season) for wild-caught, egg-bearing females. Prices for males were generally 70 to 80% lower.

Survival rates from nauplius to PL 15-20 varied from 0 to 80% and generally averaged around 25-30%. Severe mortality is often related to deterioration of water quality and subsequent disease during the rainy season. Luminescent bacteria are also cited as a common cause of mortality during the rainy season.

Government hatcheries are similar to commercial facilities in design and production capacity. They are also intended to function as non-proprietary technology transfer centres although this function could be improved.

Processing

When compared to wild-caught shrimp, farm-raised shrimp are generally rated as top quality and eligible for premium prices due to improved post-harvest handling procedures. Maintenance of shrimp quality involves keeping the product iced from harvest until the completion of processing. Loss of quality at any stage in the processing system is irreversible. At the pond site, the product from extensive farms is usually very high in quality when compared to the product from intensive farms. However, product from extensive farms is more variable in quality by the time it reaches the cold storage facility due to irregular post-harvest handling procedures.

Processing plants vary widely in age and quality control. The newer plants are better designed for producing high quality products for the export market. A typical plant has a production capacity of 5-10 metric tons per day and an ice production capacity of 5-20 metric tons per day. A weakness of some processing plants is inadequate treatment of water for processing and ice production. Some plants are exemplary in design with an airconditioned work area, ultraviolet light treated air-lock doors, tiled walls and stainless steel equipment. The better plants have well equipped quality control labs and a regular schedule for checking bacteriological and chemical quality of all major aspects of processing including receiving, product handling and water treatment. Although a variety of product forms are produced, the majority of shrimp are packaged as 2 kg headless frozen blocks.

Most shrimp processing plants require a large amount of manual labour thereby helping to alleviate rural poverty. Low labour costs in Indonesia are a major competitive advantage, particularly for further processed products such as peeled and deveined, butterflied and peeled shrimp with tails on. Most plants provide their workers with white uniforms and hats (and masks in some cases) to reinforce the concept of hygiene. Plant personnel in general appear conscientious and hard working.

Marketing

Shrimp is the largest contributor to Indonesia's fishery exports. In 1988, 71% of Indonesia's shrimp exports were directed to Japan making Indonesia the largest shrimp supplier to Japan. Other important markets include Singapore, Hongkong, Malaysia and European countries. Relatively small quantities of shrimp are exported to the US market because Indonesian shrimp is "black listed" by the US Food and Drug Administration (FDA). While it is possible for individual Indonesian processing plants to undergo FDA inspection in order to bypass the black list only about five companies have done so.

IMPACT ON THE INTERNATIONAL SHRIMP MARKET

Recent poor US domestic catches have made buyers reliant on the 80% overseas supply. The total shrimp imports increased by 5.3% in 1988. Farmed shrimp from China and Ecuador became especially prominent, with just over 20% of the market each. Despite a traditional preference for wild shrimp many traders began to appreciate farmed shrimp due to their belief that its weight and quality could be guaranteed. Similarly, the two leading shrimp markets in Europe - Britain and France - showed an increase of shrimp imports by 15% and 13%, respectively. While better preference is given to cooked and peeled cold water prawn especially in Britain, limited stocks and erratic catches are likely to cause a shift towards warm water shrimp. There have also been reports that the Dutch processors are looking for alternatives to local marine catches, whereas Spain is being flooded with shrimp from Ecuador and Argentina.

Japan, the leading shrimp importer in the world, recorded an import increase of 5% in 1988 compared to 1987, and provided a lucrative outlet for the expanding aquaculture industries, especially China and Indonesia, although the overall picture of shrimp market in Japan is not entirely encouraging. Cold storage holding at the end of January 1989 stood at 91,852 MT, nearly 10,000 MT higher than a year before which has caused a price slump. Shrimp producing countries have started to worry that the Japanese public's appetite for shrimp is approaching its limit, and unless consumption improves, there could be a disaster for shrimp price in the international markets.

Although the rapid growth of brackishwater shrimp culture has a significant impact on the increase of the world's shrimp production, there are indications that it hampered their price in the international markets.

During the spring of 1989, shrimp farmers in South East Asia watched in disbelief as the pond-side price for *Penaeus monodon* dropped from 8.50 to 4.50 US$ per kg following the slow demise of Emperor Hirohito. In Taiwan, Thailand and the Philippines, since the cost of production of shrimp using semi-intensive and intensive technology was about 5.00 US$ per kg shrimp farmers lost money on their crops. In India, Bangladesh, Vietnam and Indonesia where cheaper, extensive, technology is employed, farms remain profitable.

Although by mid July 1989 prices had somewhat improved, the price of farmed shrimp in the international market remains vulnerable to further drops. Consequently, shrimp producers in many Asian countries have cut their production which has contributed to a reduced growth rate of farmed shrimp in 1989 and in 1990.

How will the price drop of farmed black tiger shrimp in Southeast Asia affect Ecuador's industry? Fortunately, Ecuador is somewhat insulated from the Asian markets by a different product mix and its established markets, the United States.

However, with its production costs at about 3,75 US$ per kg, the next couple of years are not going to be easy for Ecuador or for any other shrimp farming country.

TECHNICAL PROBLEMS ASSOCIATED WITH BRACKISHWATER SHRIMP CULTURE INDUSTRY

In addition to strong competition in the international markets, the shrimp culture industry is also facing various technical problems which need an immediate attention.

Non-pathogenic factors

Use of high temperatures to accelerate larval growth. The ideal range of water temperature is 26 to 30°C, but hatcheries would often elevate it to 33 to 35°C to induce moulting and shorten the production period. However, although risks of larval mortality are lessened, survival rate is increased, and growth is hastened, the adverse consequences of this questionable practise eventually catch up with the prawn, when they fail to develop the resistance to natural conditions in the nursery and grow-out ponds.

Deterioration of grow-out ponds. Several years of continuous use has naturally affected the condition of grow-out ponds. Many of these ponds have been in operation for 10-15 years. But this problem is not limited to old ponds. Even new ponds may have deteriorated fast from neglect of proper pond preparation (i.e. tilling, drying of bottom soil, etc.).

Increase of stocking densities to unreasonably high levels. The carrying capacity of the ponds is often abused or overloaded. Under intensive culture, prawn are stocked at high densities, making the culture environment conductive to the outbreak of disease.

Poor choice of artificial feed. Feed accounts for 50-60% of total prawn product cost. In their effort to cut down on costs, prawn farmers tend to choose the lower priced prepared feed. Some feed manufacturers are, in turn, forced to push down their own production costs in order to offer more competitive prices but are unable to do so without affecting product quality.

Indiscriminate use of medicine and antibiotics. Antibiotics and chemicals have been applied in excessive quantities. While this may improve the prawn's resistance to disease, such immunity is temporary. Once exposed to harsh or unfavourable natural conditions in the grow-out, the prawn becomes susceptible again to infection.

Inadvertent and sometimes unavoidable use of polluted water. The absence of an efficient, well-engineered sewerage and industrial waste disposal system has made water pollution a chronic problem. Culture water can easily become contaminated with effluent, including waste material discharged by the aquaculture farms themselves. Hatcheries are especially vulnerable, since the larvae are most sensitive to water quality.

Indirectly related to this is the problem of water supply. Shrimp farmers have been competing with households and agricultural users for supply of freshwater, which they use to dilute seawater and obtain brackishwater.

Lack of technical training of farmers. This is only partly a contributing factor, but one that nevertheless demands urgent attention and action. The impressive performance of the flourishing *P. monodon* culture industry and the rising prosperity of those who had entered it encouraged a big number of enthusiastic entrepreneurs. In their eagerness to put up their own farms and rake in a profit many of them neglected to acquire basic knowledge and proper training on the technical aspects of *P. monodon* culture first, before embarking on a farming enterprise.

Absence of a reliable system of sanitation and failure to install effective sanitation systems has left hatchery facilities and grow-out ponds highly vulnerable to contamination by harmful microorganisms and has facilitated the spread of disease. Some farmers were plainly unaware, and others outright neglectful, of the possible far-reaching consequences of poor sanitary practice.

Pathogenic Factors

Under the traditional polyculture and extensive culture systems, no major problems were encountered except for the occurrence of natural disasters, and the presence of predators and competitors. As the culture systems shifted to semi-intensive and intensive styles, stocking densities were raised and formulated feeds were used. Water quality and the general culture environment became harder to manage, and culture species became more susceptible to various diseases.

Eventually, various types of infections, such as protozoan epicommensals, entozoic algae, black-gill disease, gill decay, telson damage, body cramp, and discolouration were reported to have afflicted the shrimp.

As long as culture conditions were optimal *P. monodon* appeared to be able to tolerate light to moderate infections. However, it is obvious from the above (non-pathogenic factors) that farms have failed to maintain an ideal culture environment. In many cases, outbreaks have most likely been predisposed by stress, such as poor water quality, deteriorating environmental conditions, poor nutrition, etc. Thus, it is not surprising that several pathogenic factors have been identified as part of the cause of mass mortality cases.

Quality Factor

Export of fisheries products from the Asia-Pacific region in recent years showed a sustained increase, although there were fluctuations in the supply of some commodities and from different countries. The principal export markets for fishery products are Japan, the United States and Europe. In these importing countries, consumer awareness of food safety and quality has become an increasingly important factor in the acceptability of food products. Consumer concern and media publicity about food-borne illness associated with consumption of seafoods has prompted the need for an improved sanitation and quality control systems.

Increased consumption of seafood from retail stores, restaurants and fast food outlets, together with rising product value, has placed more emphasis on higher quality products.

Over the last thirty years, authorities in importing countries with an obligation to protect the health of their citizens, have established strict quality and hygienic regulations and legislation including well defined standards for imported fishery products. In most cases the regulations refer not only to the final product but also to the establishment and the conditions under which the product is handled, processed and stored. An official health certificate for exported fishery products is most often required.

Many fish exporting countries, particularly the Asia-Pacific region, have not been able to meet such market requirements. This has led to substantial rejections of consignments and severe economic losses to the producing countries and their exporting companies. Consignments are often rejected because of contamination with pathogens and other quality defects such as poor size grading, evidence of spoilage, filth and dirt, mislabelling, etc. Although such defects might not be reason enough for an importer to reject consignments, they tend to lead to lower price offers. In fact, shrimp exports from tropical countries do not, in general, enjoy the same reputation for quality as do those from other areas. This particularly affects products from the Asia-Pacific region, with the prices of shrimp originating from the region being considerably lower than those of equivalent products from elsewhere.

CONCLUSION

The rapid growths of brackishwater shrimp culture has a significant impact on the increase of the world's shrimp production. However, there are indications that it has started to hamper their prices in the international markets leading to price slump and strong competition among exporting countries. In turn it will force the shrimp exporting countries to maintain high quality standards, otherwise they will not be able to compete in international markets. Quality defects could become a trade barrier in the future especially if the use of polluted water for shrimp farming and indiscriminate use of veterinary drugs and antibiotics becomes a common practice.

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Table 3 Projection of Future Cultured Shrimp production in Asia 1989-1999 (in '000 tons)

	1989	1990	1993	1999
Study Countries	474	560	775	1250
China	210	250	320	450
Indonesia	85	100	140	220
Thailand	75	90	110	150
Philippines	52	60	90	180
India	27	30	45	90
Bangladesh	21	25	40	100
Malaysia	4	5	10	60
Other Asia	85	50	60	80
Taiwan Province	45	50	60	80
Vietnam	30	35	45	60
Other	10	12	15	30

Source: Study team estimates

Table 4 Processing Plants for Frozen Marine Products, and Indicative Processing Costs, 1988

Country	Number of Plants	Processing Cost ($ per kg)	Processing Labour ($ per kg)
Bangladesh	93	0.63	1 - 2
China	1400	0.50	1 - 3
India	217	0.73	2 - 3
Indonesia	82	0.52	1
Malaysia	30	0.74	4 - 7
Philippines	39	0.73	3 - 4
Thailand	100	0.60	3 - 4

Table 5 Indicative Prices of Packaging Material for Frozen Shrimp

Country	Packaging Material ($ per kg of finished product)
Bangladesh	0.13
China	0.14
India	0.08
Indonesia	0.11
Malaysia	0.09
Philippines	0.10
Thailand	0.60

Table 6 Destination of Shrimp Production, 1987
('000 tons, live weight)

Country	Domestic Consumption	Net Exports	Total Production
Bangladesh	45.7	27.1	72.8
China	67.1	119.9	187.0
India	113.8	86.2	200.0
Indonesia	113.0	70.0	183.0
Malaysia	70.0	28.0	98.0
Philippines	57.8	20.0	78.6
Thailand	60.0	90.1	150.1
Percentage of Total	54%	46%	100%

Figure 1. Cultured Shrimp Production, 1984 - 1988
(in '000 tons)

	1984	1985	1986	1987	1988
Study Countries	115	150	206	310	385
China	19	41	83	153	180
Indonesia	32	37	41	52	70
Thailand	13	16	18	30	45
Philippines	28	29	30	35	45
India	14	17	19	22	24
Bangladesh	8	10	13	15	18
Malaysia	-	-	2	3	3
Other Asia	24	40	85	111	78
Taiwan Province	17	33	70	80	45
Vietnam	7	7	10	15	25
Other	-	-	5	6	8
Latin America	35	31	46	79	88
Ecuador	33	27	36	66	70
Central America	2	4	8	10	13
Other	-	-	2	3	5
Other	-	2	4	6	9
Grand Total	117	226	341	506	506

FERMENTED FISH PRODUCTS IN THAILAND; POSSIBLE DEVELOPMENT OF LOW SALT PRODUCTS WITH EXTENDED SHELF LIFE

by

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ABSTRACT

A brief survey of some aspects on fish fermentation technology in Thailand is given. The paper discusses possibilities of developing low salt fermented fish products with extended shelf life, with particular emphasis on increasing fish protein consumption among children.

INTRODUCTION

In many Asian countries fish is the major source of animal protein (FAO, 1991). In marine fisheries, as well as inland fisheries and fish farming, there are large seasonal variations in harvest volume, and the need for preservation and long term storage is urgent to maintain stable fish supply during low harvest seasons.

In Thailand, as well as in other South-East Asian countries, fermented fish products have been important food items since ancient times, and products like nam-pla (fish sauce), kapi (shrimp paste) and pla-ra (fermented fish) are still consumed daily by Thai people (Phithakpol, 1989).

Most fermented fish products contain large amounts of salt. Generally there is a growing concern about health hazard due to high salt consumption (Sakkorn Thammitore, personal communication). Hence, the high salt content limits the use of fermented fish as a protein source, and protein and energy malnutrition is apparent among Thai populations who mainly rely upon fermented fish as an animal protein source in the every day diet. Generally, malnutrition is most frequent among small children (Kent, 1987).

Traditional Thai technology for fish fermentation has been described previously (Dhamcharee *et. al.* 1982; Adams *et. al*, 1985; Saono *et. al.* 1986; Saisithi, 1987; Phithakpol, 1989) and will only be summarized briefly in this paper.

FISH FERMENTATION TECHNOLOGY IN THAILAND

The fermented fish products in Thailand can be classified into three groups (Phithakpol, 1989):

a) Fish with a large proportion of salt
b) Fish with salt and carbohydrate (mainly rice)
c) Fish with salt and fruit

All the three categories have a few things in common. They contain some kind of fish material and sea salt, and the consumption products develop during a storage period at ambient tropical temperature.

a) Fish with a large proportion of salt

The fish sauce, nam-pla, belongs to this group. Nam-pla is similar to fish sauce produced in other parts of South-East Asia, and it is the only fermented fish product which is produced on a large industrial scale.

About 80.000 tons of fish sauce is produced annually in Thailand, and this is more than twice as much as the total amount of other fermented fish products (Saisithi, 1987). The raw material may be different kinds of fish, but the best quality is obtained from anchovies *(Stolephorus sp.)*. One part of salt is mixed with three parts of fish by placing alternate layers of fish and salt in a container until it is filled up finally by placing a thicker layer of salt on top. The container is then sealed and stored for more than one year before the clear yellow to brownish fish sauce can be harvested from the top of the container. After bottling, the sauce can be stored at ambient temperatures up to one year. Nam-pla contains 22-27% salt and about 10-12% hydrolysed protein, and it has a slightly acid to neutral pH. Nam-pla has a very salty, but palatable taste and is used mainly as a condiment on rice dishes.

Partially hydrolysed fish products like shrimp paste, kapi, and fermented fish viscera, tai-pla, also belong to the high salt product group and are produced in essentially the same way as nam-pla, but the storage period is shorter (6-8 months), and the products are semi-solid pastes instead of liquids.

b) Fish with salt and carbohydrate (mainly rice)

The product variety within this group is very large. The fish used are mainly small freshwater species, and the carbohydrate is normally cooked or roasted rice.

Fermented fish, pla-ra, Thai fermented fish, som-fug, Thai anchovy, pla-chom, and Thai sweetened fish, pla-chao, belong to this group. Both salt and lactic acid produced during fermentation of carbohydrate are important preservative agents in these products. Antimicrobial compounds produced by the lactic acid bacteria may also play a role in suppressing growth of deteriorating bacteria (Lindgren and Clevestrøm, 1978; Schröder *et al.,* 1980).

Fermented fish, pla-ra, is prepared by first storing a mixture of fish and salt (10:3) in sealed jars for 6 months. Then it is mixed with roasted rice powder (1:1) and stored for a short period before it is ready for consumption. The final product is solid and has a brown or dark yellow colour. It contains equal amounts of protein and salt (about 12%) and has a slightly acid pH (5-6). The lactic acid content normally is 1-2%, and it has a shelf life of about two years.

Thai fermented fish, som-fug, is made from fish flesh, salt, cooked rice and garlic. The proportion of salt is quite low, only 1-2 parts of salt is added to 20 parts of ground fish flesh. Then 3 parts of ground cooked rice and 1 part of minced garlic is mixed in before it is packed tightly in a tray and covered. After about 5 days at ambient temperatures the product is ready for consumption. It is light, sometimes pink, and quite sour (pH 4-5 and about 2% lactic acid), contains about 15% protein and only 3,5% salt. The shelf life, however, is only two weeks.

Thai anchovy, pla-chom, is produced much in the same way as som-fug. Ten parts of fish meat is mixed with 1 part salt, 3 parts of boiled rice, 1 part garlic and 3 parts of powdered roasted rice before it is packed in jars and left at about 25°C for 3 days. The solid brown product has a sour taste. The pH is 5-6, and the lactic acid content is about 3%. It contains about 20% protein and only about 4% salt. Just like with som-fug, the shelf life is only about two weeks, and the product is therefore not very useful in maintenance of stable fish supply during low harvest seasons.

Thai sweetened fish, pla-chao, is prepared from freshwater fish, salt and khaomak, and glutinous rice which has been fermented with mould and yeast. The fish is chopped and mixed with salt (3:1), packed in closed bottles and left at 30-35°C for about 40 hours before sweetened khaomak is added to the preparation (1:1). The mixture is then stored at about 30°C for 20 days. The final product is solid, has a pink brownish colour and tastes sweet and salty. The pH is normally below 5, and the lactic acid content is 2-3%. The content of protein and salt is about 10% and 7% respectively, and this product has a long shelf life (1-2 years).

c) Fish with salt and fruit

In Thailand there are only two products which are prepared by fermenting salted fish mixed with fruit. These are khem-bak-nad which normally is prepared from striped catfish and pineapple and pla-num, which is made from snake head fish, roasted rice and papaya.

In production of khem-bak-nad, the striped catfish, which is quite large, is cleaned, bones and fat are removed and thin slices of muscle are salted (1 part of salt to 5 parts of fish meat). The salted fish is packed in containers and stored over night before it is mixed with chopped pineapple (1:1). The mixture is packed in sterilized bottles. The air is removed before the bottles are closed and left for fermentation in the dark for three months. The semi-solid consumption product, which develops during storage, is sweet, sour, salty and slightly alcoholic. It is used as a main dish and contains about 6% protein, 5% fat and 14% salt. The pH is about 4,2.

Pla-num is prepared by chopping snake head fish and mixing it with salt (3:1) before it is mixed with ground roasted rice and packed tightly in containers. After storage for 1.5-2 months the fish is mixed with chopped papaya and ground galanga (spice) and repacked in the containers. It is consumed either raw or cooked with coconut milk (Phithakpol, 1989). Little detailed information is available about this product.

Table 1 summarizes some data on the products which have been briefly described here. Both the data in the table and most of the other data presented in this chapter have been obtained from previous publications on Thai fermented fish products; Dhamcharee *et al.*, 1982; Saono *et al.*, 1986; Saisithi, 1987; Wongkhalaung and Boonyaratanakomkit, 1986; Phithakpol, 1989).

Table 1. Characteristics of some typical Thai fermented fish products

Thai name	Category	Raw material	pH	Protein/ salt ratio	Shelf life	Use
Nam-pla	High salt	Fish and salt	6-7	0.5	1 year	Condiment on rice
Kapi	"	Shrimp and salt	7-8	1.0	years (?)	Condiment on rice
Tai-pla	"	Fish viscera and salt	5-6	0.5	1-5 years	Main dish
Pla-ram	Salt and carbohydr.	Fish, salt and rice	5-6	1.0	2 years	Main dish
Som-fug	"	Fishflesh, salt rice and garlic	4-5	4.0	2 weeks	Main dish and snack
Pla-chom	"	Anchovy, salt, rice and garlic	5-6	5.0	2 weeks	Main dish
Pla-chao	"	Fish, salt and rice	4-5	1.5	1-2 years	"
Khem-bak-nad	Salt and fruit	Catfishmeat, salt and pineapple	4-3	0.5	years (?)	"
Pla-num	"	Snake head fish, salt, rice and papaya	-	-	-	"

Although the products listed in the table constitute only a part of the numerous Thai fermented fish products, the table includes some representatives from all categories.

Only two of the products listed contain considerably more protein than salt. These are pla-chom and som-fug. Both these products belong to the group; fish with salt and carbohydrate, and it is obvious that the lactic acid produced from carbohydrate during fermentation plays an important role in the preservation of these products. Since the shelf life of both products is only two weeks, it is evident that they are not suitable as a fish protein supply during low harvest seasons. All the products with long shelf life contain high amounts of salt, and the protein/salt ratio is poor.

Although high salt products like fish sauce and fish paste are used mainly as condiments, these products yields more than 100.000 tons a year, which is far more than the amount of other fermented fish products made in Thailand (Saisithi, 1987). Even if some of the fish sauce is exported, it is obvious that these products are important protein supplements for a large part of the Thai people.

The annual production of other fermented fish products is about 13.000 tons (Saisithi, 1987). These products are eaten as main or side dishes, and they are an important animal protein source for the rural population in many parts of Thailand.

NUTRITIONAL ASPECTS OF FERMENTED FISH PRODUCTS

Although Thailand at present has a vigorous economic development, the protein energy malnutrition is widespread, and particularly among small children. In a survey of one million children under five, conducted in 1980-81, 35% were mildly malnourished, 13% were moderately malnourished and 2% were severe malnourished and about one-fifth of the schoolchildren in the Nonhem region suffered from iodine deficiency (Kent, 1987).

In Thailand fish accounts for about half of the protein supply and 14% of the total protein consumed, and the average per capita consumption of fish is about 15 kg per year (Kent, 1987).

Fish protein has an amino acid composition that complements the imbalance of cereal proteins, particularly by contributing with high levels of essential amino acids like lysine and methionine. Fish also contains a number of important vitamins as well as minerals and iodine. Generally fermented fish prepared properly contains most of the same valuable nutrients as fresh fish. However, some amino acids like arginine and tyrosine may be susceptible to degradation by lactic acid bacteria, and an average amino nitrogen loss of about 30% has been recorded during fermentation of traditional products (Amano, 1962). The oil in fermented products has a low nutritional value due to rancidity, and the vitamin A content decreases rapidly during fermentation (Amano, 1962).

Low salt products like pla-chom and som-fug have a comparatively high protein/salt ratio, and may therefore be considered as high value nutritious food if properly prepared under hygienic conditions. However, since these products have a very short shelf life, they are not useful to extend the protein supply during low harvest seasons.

High salt products like pla-ra and nam-pla may be stored for at least one year and can therefore serve as a source of animal protein during all seasons. The high level of salt in these products, however, limits the advisable daily intake.

The daily requirement of NaCl by an adult is about 2 g. This level would be reached by consumption of about 16 g pla-ra or 8 g nam-pla, corresponding to about 2 or less than 1 g protein respectively. However, the total salt consumption, at least in the tropical regions, may be as high as 10 g per day without causing health problems like high blood pressure and cardiac diseases, which are the main adverse effects of too high salt intake.

Since the daily protein requirement is about 1 g per kg body weight per day, it is obvious that only a very small fraction of the requirement can be supplied from high salt fermented fish products. Larger amounts can be consumed of the products fermented with rice, but these products have a very limited shelf life and can not serve as a protein supply during low harvest seasons. Development of low salt fermented fish products with extended shelf life is necessary if fermented fish shall function as a protein supply during the whole year.

The fermented fish products with a low or medium range salt content are typically semi-preserved food products. The major potential health hazard associated with such products is growth of food poisoning bacteria, presence of parasitic worms and the production of physiologically active amines. Of particular concern with non-heated foods offering anaerobic conditions, is the possible growth and toxin production by *Clostridium botulinum* (Lee, 1990). To avoid such problems it is important to prepare the products from fresh raw material under proper hygienic conditions. These requirements may not always be easy to fulfil, because these products are mainly prepared at a home industry level in rural areas. Thai food researchers have implemented consultancy projects to give advice to rural village societies in North-Eastern Thailand to improve the safety and quality of fermented fish products (Prapasri Puwastien, personal communication).

Although not very useful for ordinary people, data which state the limits for growth of the major hazard microorganisms are important for experts giving advice, and for researchers in their work with development of new products. Table 2 summarizes data concerning pH, water activity and salt concentration.

Table 2. Maximum sodium chloride concentrations and minimum pH values and water activities for growth of food poisoning bacteria under otherwise optimal conditions (modified after Owens and Mendoza, 1985).

Bacteria		Maximum salt %	Minimum pH	Minimum water act.
Bacillus cereus		7.5	4.4	0.94
Clostr. botulinum				
	types A and B	10	4.8	0.94
	type E	5	5.0	0.97
Clostr. perfringens		6	5.0	0.95
Staphyl. aureus				
growth:	aerobic	18	4.3	0.86
	anaerobic	16	4.7	0.90
toxin:	aerobic	13	4.3	0.90
	anaerobic	13	6.5	0.90
Vibrio parahaemolyticus		10	4.8	0.94
Salmonella sp.		8	4.1	0.95
Listeria monocytogenes		10	4.7	-

Generally the data in Table 2 show that most fermented fish products should be quite safe if prepared according to recommended procedures. In the high salt products, the salt alone prevents the growth of hazardous microorganisms, whereas the low pH or the low pH combined with salt prevent growth in the carbohydrate fermented products. However, toxin producing moulds like *Aspergillus flavus* may grow even at a very low pH on air exposed surfaces.

DEVELOPMENT OF NEW LOW SALT PRODUCTS TO INCREASE FISH CONSUMPTION PARTICULARLY BY CHILDREN

As already shown (Table 1), some traditional fermented fish products have quite low salt content. However, these products have a low storage stability and often a strong acidity that may inhibit consumption by small children.

Salt is the main preservative agent in most fermented fish products. Significant salt reduction would imply health hazard if not compensated by other preservative means. The main risk factor is growth of pathogenic or toxin producing microorganisms. In addition to the salt concentration, the pH and the water activity are important factors which may restrict growth of microorganisms (Table 2). Introducing antimicrobial chemical compounds is another way of restricting microbial growth. In fermented fish products we want to prohibit the growth of dangerous microorganisms and promote the growth of lactic acid bacteria. However, it is also important to avoid too high lactic acid production, which may reduce acceptability particularly among small children.

First of all we have to make a choice of what kind of products we wish to develop. From the previous chapters we know that the flora of fermented fish products is already very diversified. The most obvious question to ask would be: what does the consumer, and particularly the low age consumer, prefer? An investigation performed in North-East Thailand by the Institute of Nutrition at Mahidol University has shown that the children started to eat cooked or baked fresh fish before the age of one year, whereas they normally did not start to eat fermented fish before at the age of about two years. The investigation also showed that fresh fish was preferred if available (Prapasri Puwastien, personal communication).

One conclusion that may be drawn from this is that if we shall use fermentation technology to develop products with high acceptability among children, we should try to make them as similar to fresh fish as possible. In other words, we should try to preserve the fresh fish quality as much as possible, and use the fermentation mainly as a preservation principle.

An alterative way of introducing fish nutrients to children is by using fish preparations as ingredients in snack products like sweets or cookies. The Fishery Technology Division of the Department of Fisheries has been experimenting with fish crackers, fish noodles and fish biscuits based on fish protein concentrate (FPC). Acceptability tests with fish biscuits containing as much as 15% FPC have given promising results among schoolchildren in Thailand (Charoenphol *et al.*, 1991). The FPC, however, is produced by a rather advanced and expensive process, and there is an obvious need for development of easier and cheaper methods to produce fish protein preparations suitable for such application. Fermentation or enzyme hydrolysis could be such alternatives.

Some of the traditional fermented fish products like for instance som-fug or fish fermented with fruit may be suitable as ingredients in snack products. They have an aromatic flavour and a relative high protein content and may improve the nutritive value of the snack products significantly even if relatively small amounts are added.

If fermentation is to be used to preserve the freshness of fish, there are a number of factors to consider. First of all we should be aware of the factors which change freshness into something else. These factors may be categorized into three groups:

- chemical additives
- microorganisms and their products
- enzymes present in the fish material

In fermented fish products the major chemical additive is sea salt. Heavy salting has a strong implication on the fish quality. The immediate consequence of salting is extraction of water and salt water soluble compounds from the fish meat implying a tougher consistency of muscle tissues. Then the salt successively penetrates the fish tissues and the toughness decreases due to water uptake. However, the fish is not fresh any more. It is salty and has lost water soluble compounds as well as a part of the structural proteins that is salt soluble. Such compounds may be collagen from the connective tissue and myofibrillar proteins from the muscle. To retain optimal freshness of the fish, it is therefore important to keep the salt concentration at a level as low as possible without risking growth of food poisoning bacteria. This level is of course impossible to quantify by an exact value, but must be considered together with other growth restricting parameters like pH, temperature and water activity.

Among the microorganisms in fermented fish products, the lactic acid bacteria are, or at least should be, the major group. The most important function of the lactic acid bacteria is to produce lactic acid, which normally is the second of the two main preservative agents in fermented fish products. In addition the lactic acid bacteria also produce chemical compounds called bacteriocines. These compounds inhibit the growth of proteolytic bacteria which may result in rapid deterioration and putrefaction. While the lactic acid bacteria degrade protein only to a minor extent, the proteolytic bacteria vigorously attack protein and produce ammonia and toxic amines. Hence, for the means of preservation and retainment of freshness during fish fermentation, it is important to select lactic acid bacteria with a high rate of bacteriocine production.

In some traditional fermented fish products like for instance som-fug, the lactic acid production is very high, however, the shelf life is short. In this case short shelf life does not always mean that the product deteriorates rapidly. Sometimes it rather means that the product becomes too sour to be acceptable to the consumers. This may imply that an extended shelf life could be obtained if lactic acid bacteria with a moderate acid production and high bacteriocine production were used. This means that addition of a defined lactic acid bacteria culture would have to be one step in the production procedure. This would implicate a modification of traditional methods, since these are based on utilization of the lactic acid bacteria naturally occurring in the raw materials. However, if such a modification could imply a considerably extended shelf life, it would certainly justify some extra labour and expenses.

Generally strong acidity changes the fish muscle structure significantly. The muscle fibres contracts transversely and the fish meat loses water and gets a tough dry appearance not compatible with fresh fish quality.

To retain optimal freshness it is evident that both salt and acid concentration must be kept at a moderate level. This obviously must mean some loss of preservative effect which has to be compensated by other preservative means that are less detrimental to the fresh fish quality. There are a large number of chemical compounds which may restrict the growth of deteriorating microorganisms, but for this particular purpose the compounds must fulfil many requirements. They must not be harmful for the consumers, must not give adverse flavour to the product, must not inhibit the growth of lactic acid bacteria and finally, as already mentioned, must not cause structural or textural changes which destroy the fresh appearance of the fish muscle. All these requirements reduce the number of actual candidates considerably.

In Norway promising results have been obtained by addition of moderate amounts of sorbate and benzoate to stabilize fermented fish. These compounds are legal additives to fish products in Europe. It is advisable to use the potassium salts to counterbalance the already high level of sodium due to salt addition. Both these compounds are extensively used as preservatives in the food industry, and to the authors knowledge, no harmful effects have been documented at moderate levels (below 1%).

In China extracts of polyphenolic substances from tea processing waste have been used as preservatives in fish sauce. About 200-300 ppm were added without any adverse effects on the flavour of the fish sauce (Shi Xian-Quan, personal communications). The polyphenolic substances can also be sprayed on the surface of other fermented fish products and may cause extended shelf life.

Some spice extracts also have inhibitory effect on the growth of putrefying bacteria. Such spices may be ginger, nutmeg, rosemary, sage, black and white pepper (Shi Xian-Quan, personal communication). Some substances extracted from spices are called essential oils. Such essential oils have no colour and a pleasant flavour and have high inhibitory effect on putrefying bacteria. However, the price is high and such products are only used in very refined fermented fish products. It is also well known that garlic has a strong inhibitory effect on many putrefying bacteria (Souane *et al.,* 1990), and it is used in many traditional fermented fish products both in Thailand and other South-East Asian countries. It has been claimed that garlic juice in concentration as low as 1/80.000 was lethal to typhoid and cholera bacteria (Kato, 1973). Spice extracts obviously would have an effect on the flavour of the product, but would probably not change the functional properties of the fish muscle.

Fish muscle contains significant amounts of proteolytic enzymes. Particularly so, if the fish has not been eviscerated immediately after capture. Digestive proteases like pepsin, trypsin and chymotrypsin rapidly leak out from the digestive tract and penetrate the muscle tissue. At neutral conditions trypsin and chymotrypsin attacks the myofibrillar proteins and cause tissue softening and loss of protein material into the soluble phase. Under acid conditions pepsin from the stomach and cathepsins, which are naturally present in the muscle cells, may cause significant autolysis and structural deterioration of the muscle tissue. Autolysis is one of the major causes of fish deterioration and it also results in considerable loss of tissue proteins into the aqueous soluble fraction. Autolysis may be limited if the fish is eviscerated and washed immediately after capture. This will minimize the entrance of digestive enzymes into the muscle tissue. However, the cathepsins which are proteolytic enzymes active at weak acid conditions, will always be present in the muscle and may cause significant structural deterioration in fermented fish products which normally have a pH which is just about optimal for their activity.

The enzymes may be inactivated by heating, but this obviously would destroy the fresh appearance of the fish tissue. The action of the enzymes may, however, be reduced to a minimal level by addition of enzyme inhibitors. Such inhibitors are chemical or biochemical compounds which specifically block the active site of the enzymes. Different inhibitors exist for different enzymes, and sources of such inhibitors are various. Many plants are rich in inhibitors against proteolytic enzymes, and best known is probably the trypsin inhibitor that can be extracted from soy beans. Small amounts of these inhibitors very efficiently block the activity of both trypsin and chymotrypsin. Inhibitors with a similar effect are present in lima beans and potatoes. Also blood plasma contains considerable amounts of inhibitors against tryptic activity.

Since fermented fish products generally are acid products, inhibitors against pepsin and cathepsins may be of particular interest. The cathepsins are a diversified group of enzymes with few common characteristics except for the pH optimum range which is acid to weakly acid. The major muscle cathepsin, however, is cathepsin D, and this enzyme is very similar to pepsin. Both enzymes belong to the group of aspartic proteinases. Only a few naturally occurring inhibitors against aspartic proteinases have been detected. Best known is pepstatin which is a small peptide of microbial origin. This inhibitor is very efficient, but probably not to be recommended as a preservative agent in consumption products. It is also very expensive to produce.

It has been shown that the seeds and shell from the tropical fruit tamarind are rich in inhibitors against aspartic proteinases, and experiments performed in Norway with fermented herring have shown that tamarind fruit or extracts from milled shells and seeds from tamarind may reduce autolysis of the fish muscle and contribute to preserve the native structure of the tissue (Andersen, 1984). In these experiments herring fillets were stored for 30 days at about 20°C in a weak salt solution (5%) containing various other preservatives like sorbate, benzoate and ascorbate in addition to different amounts of tamarind fruit. Some of the samples retained a very good structural and organoleptic quality through the whole storage period.

The tamarind tree grows in tropical regions with a fairly dry climate and is common in many places in Africa, India and South-East Asia. The legume fruit weighs about 12 g and seeds and shell yields more than half of the total weight. The inhibitors are both heat and acid stable, and can probably be most efficiently extracted by stirring powdered shells and seeds in a hot weakly acid aqueous solution.

Proteinase inhibitors in fruits are part of the defence system against proteolytic microorganisms, and undoubtedly many other fruits may contain similar inhibitors, however, so far the research in this field has been rather sparse, and much work remains to be done.

From the considerations in this chapter, it may be concluded that measures can be taken to retain a fresh appearance of fish muscle during fermentation storage. These measures can be summarized as follows:

- Washed fillets with skin should be immersed in a minimum amount of fermentation solution containing a cheap source of readily fermentable carbohydrate.

- The salt and acid level should be limited to reduce salt extraction and acid denaturation.

- Lactic acid bacteria with high production of bacteriocines inhibiting the growth of putrefying microorganisms should be used as a starter culture.

- Some preservatives like sorbate, benzoate and ascorbate may be added to prevent mould growth and rancidification.

- Naturally occurring preservatives of plant origin could be introduced.

- Proteinase inhibitor preparations from plants could be added to reduce autolysis.

- The fermentation container should be filled up, closed and stored in the dark at the lowest possible temperature under the given circumstances.

By the end of the storage period, the fillets should be washed to remove salt and prepared in the same way as fresh fish. The fermentation liquid could be utilized as a fish sauce. More salt should be added to secure full preservation, and the solution should be boiled and filtered before bottling. A similar procedure is used traditionally during Pla-ra preparation. It should be emphasized, however, that fish sauce prepared as described above, probably would have a rather low protein content and would therefore mainly have value as a condiment.

Preparation of fish protein hydrolysates by enzyme hydrolysis can scarcely be defined as fish fermentation. It should be mentioned, however, that such methods have been developed during several decades, and that a large number of procedures have been established. Generally the technology is quite simple. Enzyme and water is added to chopped or minced fish. Normally equal amounts of fish and water are used. The hydrolysis is performed with stirring and heating for a rather short period (0.5-2 hours). If the hydrolysis period is too long the hydrolysate becomes more bitter. After the hydrolysis the hydrolysate is normally heated to 80-90°C to inactivate enzymes and pasteurize the product which is then filtered, evaporated and often spraydried. Such fish protein hydrolysates retain almost the same nutritive value as fresh fish and may be a valuable protein supply in other less nutritious products. If prepared in the right manner, the fish protein hydrolysates have only a mild flavour and may be added to other products without giving an adverse taste. Such hydrolysates can be made from small low price by-catch fish and be an interesting alterative to roller dried fish (RFD) or the rather expensive FPC presently being used in various snack products for children (Phithakpol *et al.,* 1988; Charoenpol *et al.,* 1991).

Efforts should be made to develop new snack products with pleasant fish taste and high fish protein concentration. Such products, however, would probably need governmental subsidies to become available as a protein supply to the most indigent consumers.

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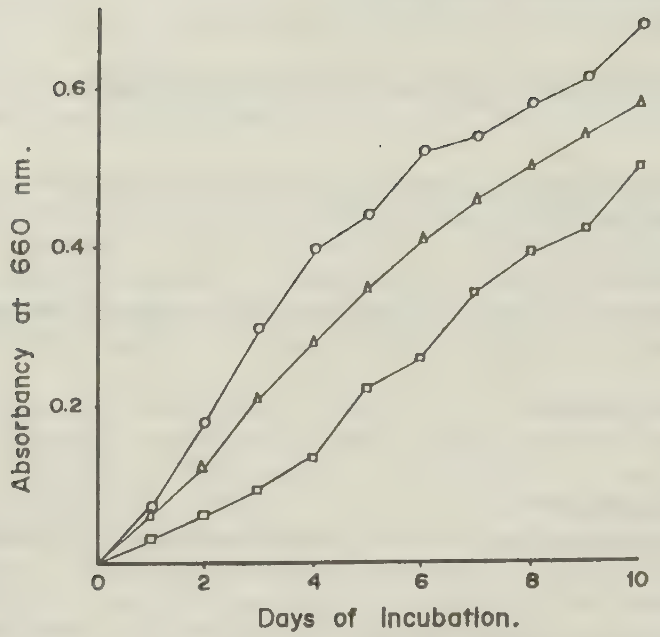

FIGURE 1. Effect of temperature on growth of Halobacterium OR.

Symbols : ○ = 37°C ; △ = 45°C ;

□ = Room temperature (28° - 32°C)

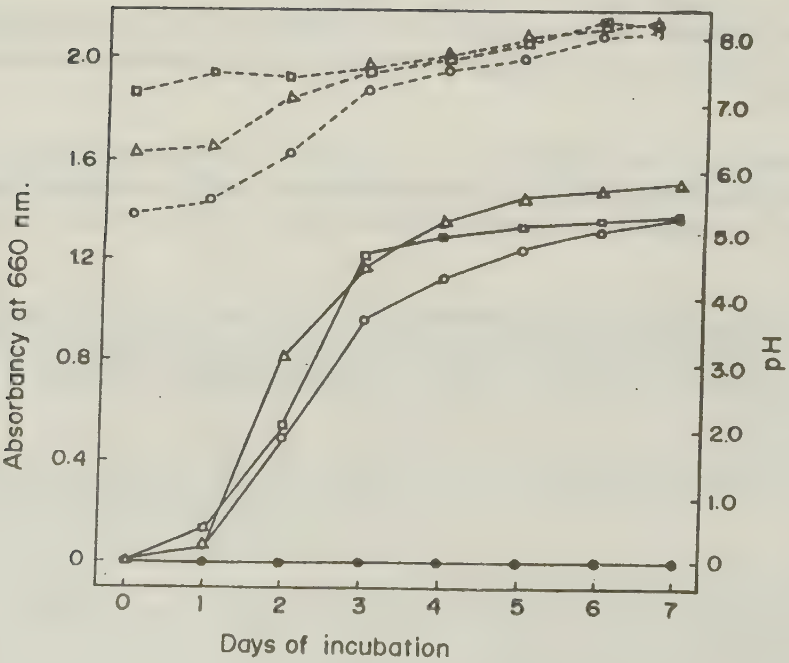

FIGURE 2. Effect of pH, growth of Halobacterium OR.

Symbols: Starting pH : ○ = 5.5 ;

△ = 6.5 ; □ = 7.5

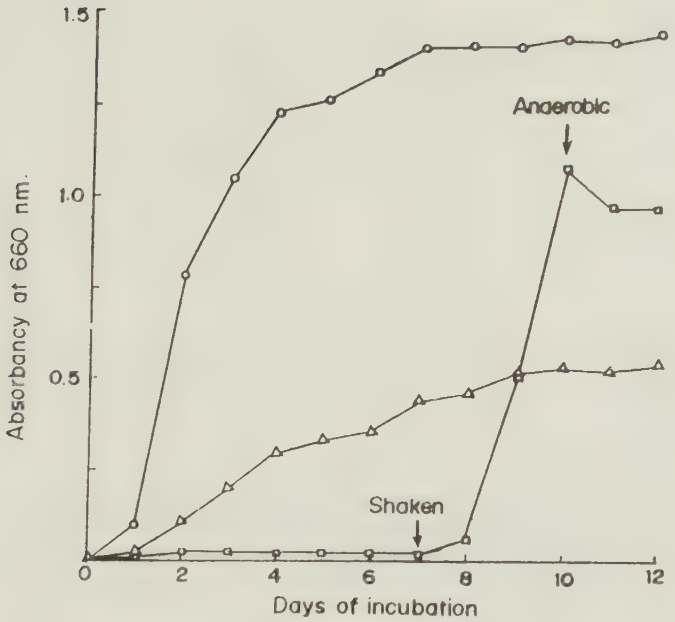

FIGURE 3. Effect of aeration on growth of *Halobacterium OR.*

Symbols : ○ = shaken at 200 rpm. ; △ = Static ;
□ = anaerobic jar.

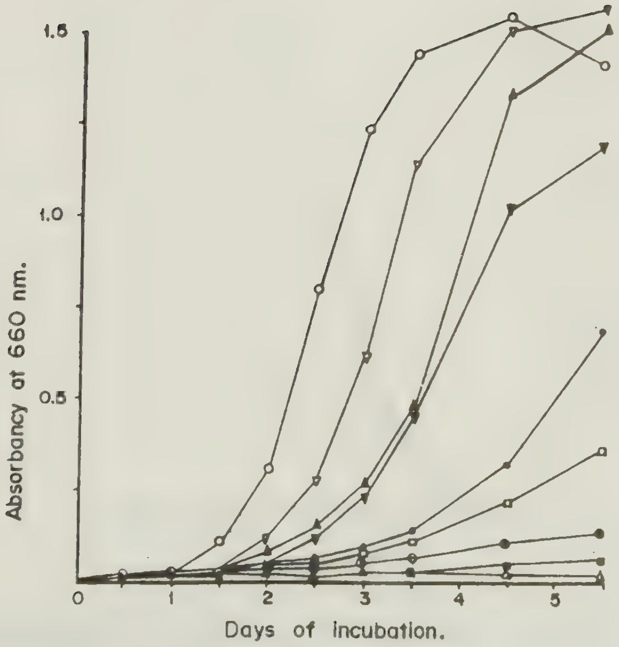

FIGURE 4. Effect of amino acids on growth of Halobacterium OR.

Symbols : ○ = CDM ; ▽ = -Thr ; ▲ = -Phe ; ▼ = -Tyr ; ● = -Glu ;
□ = -Ile ; (●) = -Met ; ■ = -Pro ; △ = -Val,-Arg,-Lys,-Leu.

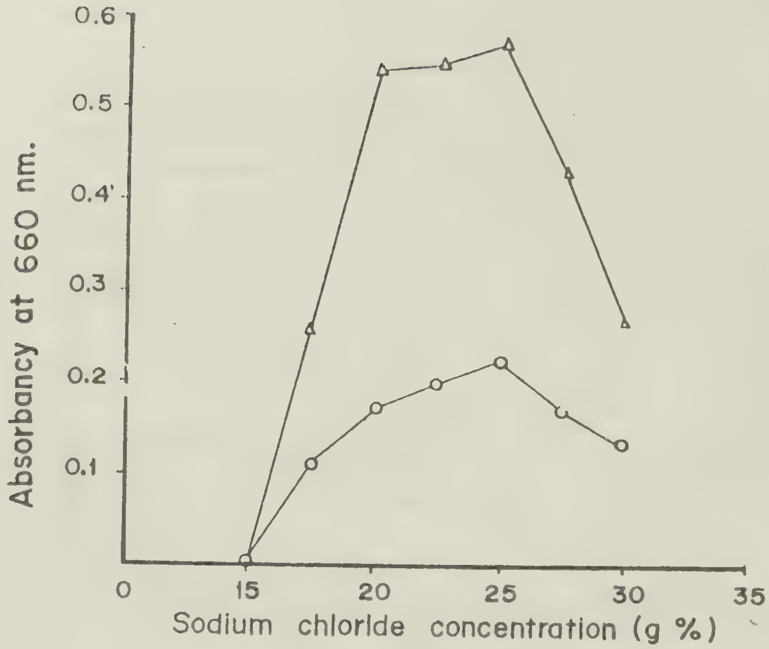

FIGURE 5. Effect of Sodium chloride on growth of *Halobacterium OR.*

Symbols : ◯ = cultivation in CDM for 48 hours.

△ = cultivation in CDM for 60 hours.

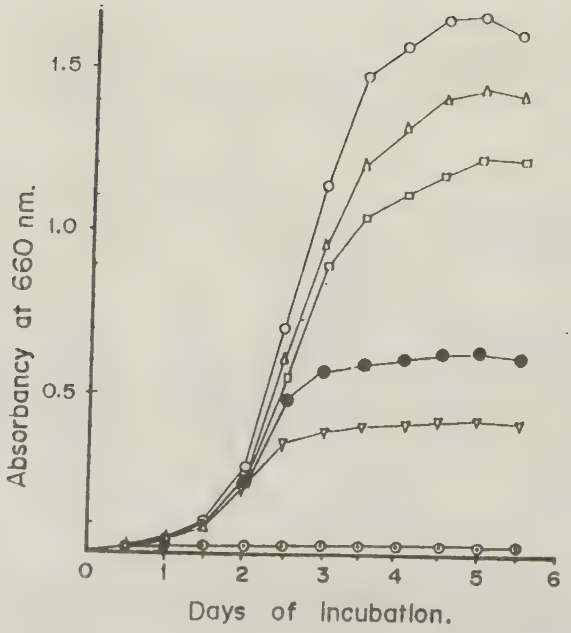

FIGURE 6. Effect of potassium ion on growth of Halobacterium OR

Symbols : ⦿ = 0.00 μmole K^+/ml. ; ▽ = 1.94 μmoles K^+/ml.

● = 2.56 μmoles K^+/ml. ; △ = 12.79 μmoles K^+/ml.

◯ = 25.58 μmoles K^+/ml. ; □ = 51.16 μmoles K^+/ml.

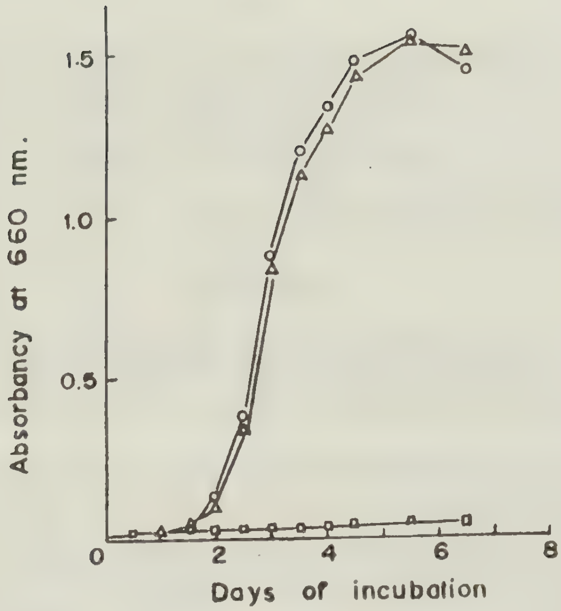

FIGURE 7. Effect of phosphate ion on growth of Halobacterium OR.

Symbols : □ = 0.00 µmole $PO_4^=$/ml.

○ – 0.65 µmole $PO_4^=$/ml.

△ = 1.30 µmoles $PO_4^=$/ml.

FISH SAUCE FERMENTATION: ROLES OF MICROORGANISMS

by

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ABSTRACT

Halobacterium sp were found most abundantly among specimens of fish sauce, fish residue and marine salt; therefore, study of the nutritional requirements of this microorganism may shed some light on its role in the fermentation of fish sauce.

The *Halobacterium* strain *OR* was originally isolated from a fish sauce specimen one month old. Spectrophotometric and viable colony count studies revealed that *Halobacterium OR* is an auxotroph for four amino acids, i.e., arginine, lysine, leucine and valine. There area also six growth stimulating amino acids, glutamic acid, isoleucine, phenylalanine, proline, tyrosine and methionine. The organism was also growth dependent on NA^+, Cl^-, K^+ and $PO^=_4$ at the respective optimal concentrations of 4.279, 4.275 and 0.00065 mM per ml. Since all growth requirements of *Halobacterium OR* were met by natural composition and fermentation conditions in the fish sauce, it was concluded that at least growth of *Halobacterium* was indigenous in, if not indispensable to, traditional fish sauce fermentation process.

INTRODUCTION

Fish sauce is a popular condiment among the Southeast Asian population and is prepared from fish in concentrated brine. The clear brown liquid, tasting salty with a cheesy aroma is a rich source of protein, amino nitrogen, iron, calcium, B vitamins (Campbell-Platt, 1987), especially vitamin B_{12} (Areekul, *et al.* 1974). Similar products are known locally as nam pla (Thailand), nuoc mam (Vietnam), patis (Philippines) and ketjab ikan (Indonesia) (Mackie *et al.* 1971). Traditionally, nam pla is made by mixing two parts fish with one part marine salt. The mixture is put into a large vat, covered with concentrated brine and left to ferment, undisturbed for up to twelve months, when it is ready for consumption.

Saisithi *et al.* (1966) found several halophilic bacteria in nam pla, and suggested that they may be important in aroma production. Crisan and Sands (1975) identified several species of Bacillus from fish sauce. Thongthai and Siriwongpairat (1989) demonstrated that there were at least two bacterial populations present in a traditional fish sauce fermentation process. The major population consisted of red extremely halophilic bacteria, identified as *Halobacterium salinarium* (Thongthai *et al.* 1991). This population reached maximum density in the liquor after three weeks and decreased thereafter to undetectable concentration after about six months. The minor bacterial population was heterogenous and halotolerant or moderately halophilic (Thongthai and Suntinanalert, 1991). The red archaebacterial halobacterium was found most abundantly among specimens of fish sauce, fish residue and marine salt (Suntinanalert, 1979). The study of the nutritional requirements of this microorganism may shed some light on its role in the fermentation of fish sauce.

MATERIALS AND METHODS

Halobacterium OR strain was originally isolated from fermenting fish sauce liquor and maintained in lyophilized form. The organism was identified to be *Halobacterium salinarium* strain OR (Thongthai *et al.* 1991).

Media. Maintenance medium was of HMB (Halobacterium Medium Broth) which was composed essentially after medium 213 of American Type Culture collection Catalogue of Strain I, 12th edition,

1976. It contained 25% NaCl, 1% $MgSo_4.7H_2O$, 0.5% KCl, 1% yeast extract and 0.25% Tryptone. To solidify, 2% agar was added.

Composition of chemically defined medium was modified from Onishi's (Onishi *et al.* 1965): Amino Acids (mg per 100 ml.); L-alanine, 43, DL-Arginine, 40, L-cystine, 5, L-glutamic acid, 130, glycine, 3, DL-isoleucine, 44, L-Leucine, 80, L-Lysine, 85, DL-Methionine, 37, DL-phenylalanine, 26, L-proline, 5, DL-serine, 61, DL-threonine, 50, L-tyrosine, 20, DL-valine, 100; inorganic salts (mg per 100 ml); NaCl, 25,000 (25 g) $MgSO_47H_2O$, 0.03, $CaCl_2.2H_2O$, 0.7, $ZnSo_4.7H_2O$, 0.044, $FeCl_2$, 0.233, $CuSO_4.5H_2O$, 0.005, KCl, 176.17; vitamins (mg per 100 ml); p-amino benzoic acid, 0.026, nicotinic acid, 0.5, Thiamine hydrochloride, 0.006; nucleoside (mg per 100 ml); uridine, 10 and other carbon source (mg per 100 ml); dextrose, 200, glycerol, 100, and sodium citrate, 500.

Methods. Growth in liquid media was measured by using a spectrophotometer at 660 nM. Viable bacterial count was done by plating of specimens on HMA and incubated aerobically at 37°C for up to fourteen days.

Variations of growth conditions were based on three parameters: temperature, pH and aeration, to obtain the optimal growth condition.

Chemical requirements for growth of *H. salinarium* OR was derived by using the Modified Chemically Defined Medium of Onishi *et al.* (1965) as a reference growth medium against growth in an identical medium missing a single component.

RESULTS AND DISCUSSION

Effect of Temperature

Halobacterium salinarium OR is a mesophyllic organism. Growth at 37°C was shown to be better than at higher (45°C) or lower (28-30°C) temperatures (Figure 1). Though higher temperature depresses growth only slightly, lower temperature retarded growth more markedly. This was hardly surprising since the species had a long history of associations with sunlight, being first discovered in the salt lakes and salterns where high concentration of salt was found (Grant and Larsen, 1989).

Effect of pH

Results of experiments on growth of *H. salinarium* OR at between pH 4.0 to pH 8.0 showed that the organism only grew between pH 5.0 and 7.5 (Figure 2). Regardless of the initial pH, the final pH of the medium, after which growth had reached a stationary phase, was 8.2 when no further growth was measurable. Therefore, for batch culture of OR, suitable starting pH was between 6.0-7.0).

Effect of Aeration

Figure 3 had shown clearly that strain OR was strictly aerobic. No growth was observable when oxygen was excluded from the environment, despite its survival ability probably on arginine (Hartmann *et al.* 1980) provided in the medium.

Nutritional Requirements

With some modification the chemically defined medium of Onishi *et al.* (1965) was used essentially for derivation.

Cultivation of *H. salinarium* OR in the originally chemically defined medium of Onishi raised the optical density maximally to only 0.45 units. However, with some modification, it was possible to raise the O.D. up to 1.6 units. By using the forgoing optimal growth conditions, and the composition of this modified chemically defined medium, omitting each time a single constituent, results of strain OR cultivation are shown in Figure 4. *Halobacterium salinarium* strain OR was auxotrophic for four amino acids, arginine, leucine, lysine and valine. Six other amino acids were growth promoting: they were proline, methionine, isoleucine, phenylalanine, tyrosine and glutamic acid. Growth rate was depressed substantially when any of these amino acids was missing from the medium. Doubling time of OR strain increased from 6 h 40 min in the complete CEDM, to 10 h, 12 h 6 min. and 10 h 56 min. in CDM lacking glutamic acid, phenylalanine and tyrosine, respectively. Except for arginine, proline and glutamic acid, all the other amino acids remained detectable after growth had reached the stationary phase. However, growth did not resume even when arginine, proline and glutamic acids were supplied at twice the original concentration. Therefore, exhaustion of these three amino acids was not growth limiting at this point.

Beside amino acids, the strain OR exhibited specific requirements for extremely high concentrations of Na, Cl, K and phosphate ions. As expected, growth of *H. salinarium* OR required NaCl in an excessively large amount. Growth was maximal at 25% (4.3 M) concentration, retarded below 20% (3.4 M) and was totally inhibited below 15% (2.5 M) (Figure 5). Although K^+ concentration for growth in the CDM was only about 25.85 mM. (Figure 6). the K^+ was required for intracellular enzyme stability (Kushner, 1985). Figure 7 shows the optimal requirement for phosphate ion of the strain OR to be 0.65 mM. No significance in growth was observed, when the amount of phosphate was doubled in the medium. While glycerol appeared to be growth-stimulating for *H. salinarium* OR, dextrose was non-contributing. These findings agreed with Gochnauer and Kushner (1969) on the *Halobacterium* species. Uridine, p-amino benzoic acid, nicotinic acid and thiamine hydrochloride and sodium citrate were not required for cultivation of strain OR; hence, they should be omitted from the CMD along with dextrose.

CONCLUSION

This study showed that four amino acids and a large amount of sodium chloride were absolute necessities for growth of *Halobacterium salinarium* strain OR. Earlier findings showed that strain OR was both gelatinolytic and caseinolytic (Suntinanalert, 1979 and Thongthai and Suntinanalert, 1991); also appreciable salt resistant protease activities were found in the fermenting liquor up to four weeks or so of fermentation (Thongthai *et al*, 1990) supported a role in peptide hydrolyses in fish sauce fermentation for *H. salinarium* OR. Further, the saturated salt concentration and abundant availability of fish protein and amino acids occurred in the fermentation vat where the strain OR was originally isolated, and provided a good support for its growth and survival, for at least until the exhaustion of its pools of arginine (Hartmann *et al,* 1980).

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INFLUENCE OF COMMERCIAL STARTER CULTURES ON THE RATE OF RICE-FISH FERMENTATION

by

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ABSTRACT

The influence of commercial starter cultures on the rate of rice-fish fermentation was investigated. Commercial lactic starters used were Lacto-start (*Lactobacillus plantarum*), Pedio-start (*Pediococcus cereviseae*), and a mixture of the two starters. Microbiological counts, pH, and total titratable acidity were monitored daily from 0 to 7 days. Proximate, salt, and sensory analyses were done on the final products.

Results revealed that the addition of commercial starters increased the rate of fermentation as measured by significant increases in MRS count (10^7) and lower PCA counts (10^6). Maximum ratio of MRS to PCA counts were attained after 24 hours. pH decreased to 4.5 or lower in 24 hours and 1.03 to 1.42% lactic acid content was achieved within a week.

Proximate composition and salt content of the products treated with starters were comparable with samples prepared traditionally. Sensory analysis likewise revealed that samples with starters were comparable with samples without starters in odour, texture, flavour, and general acceptability. Use of starters accelerated fermentation without bringing about adverse effect on flavour and texture.

INTRODUCTION

"Burong isda" is a fermented mixture of salt, boiled rice, and fish. Several microorganisms have been isolated in "burong isda" prepared traditionally. Orillo and Pederson (1968) isolated strains of lactic acid-producing species like *Leuconostoc mesenteroides, Pedicoccus cereviseae, Lactobacillus plantarum, Streptococcus faecalis,* and strains of *Micrococcus* in "burong dalag".

The role of yeasts in flavour and quality development in "burong isda" was studied by Sakai *et al.* (1983). Dominant yeasts isolated were *Saccaromyces cereviseae, Candida tropicalis, Pichia vini*, and *P. strasburgensis.* It was concluded that the presence of yeasts was due to chance contamination.

In a related study, Sakai and Caldo (1983) obtained 249 isolates from four samples of red "burong isda" and revealed that 69% of the isolates were homofermentative rods producing D-lactic acid.

Pederson (1971) revealed that a sequence of growth was necessary to reduce complex food to simple elements. Mabeza (1983) observed that fermentation showed a sequential lactic acid growth in "burong isda" prepared the traditional way. It was started by a low-acid tolerant *Streptococcus*, followed by a medium-acid tolerant *Leuconostoc*, and completed by the high-acid tolerant *Pedicoccus.*

A few studies have investigated the use of pure culture in fish fermentation. Mabeza (1983) used pure cultures of *Pedicoccus acidilactici* and *Leuconostoc paramesenteroides* as bacterial starters. Results revealed that a mixture of the two organisms yielded better quality of "burong bangus". Likewise Adams (1987) used *Lactobacillus plantarum* and *Pediococcus plantarum* and *Pedicoccus pentosaceus* in a minced fish-salt-glucose model system.

According to Stanton and Yeoh (1977), lactic acid bacteria comprise a small fraction of fish microflora. This finding suggests the usefulness of using starters of microbial cultures of effect a desired fermentation in fish products (Adam *et al*, 1986). This study therefore aimed to determine the influence of commercial starter cultures on the rate and progress of rice-fish fermentation.

MATERIALS AND METHODS

Preparation of "Burong Tilapia"

Tilapia (*Tilapia nilotica*) was descaled, gutted, and the skin was rubbed with salt to remove surface slime, then washed and drained. The fish was split in half and cut into 20g pieces. The pieces were soaked in 10% brine (1:1 w/v) for 30 minutes and then washed and drained. Sixty percent of the required salt was rubbed on the pieces and allowed to drain overnight. Another 20% of the required salt was mixed in and 0.05% starter cultures were added. Starter cultures (Chris Hansen) used were Lactostart (*Lactobacillus plantarum)*, Pediostart (*Pediococcus cereviseae*), and a mixture of the two starters. The rice and fish were arranged alternately and packed tightly into self-sealing bags. Then the bags containing the mixtures were placed in sealed plastic containers and allowed to ferment for seven days.

Using the method above, eight individual lots per treatment were prepared to represent 0 to 7 days of fermentation since microbiological counts, pH, and total titratable acidity were monitored daily. However, for proximate composition, salt and sensory analysis, several samples per treatment were prepared in bulk and fermented continuously for 7 days.

Microbiological Analysis

Lactic acid bacteria were enumerated on deMan Rogosa Sharpe Agar (MRS) while other bacteria were enumerated on plate count agar (PCA). The plates were incubated at 32°C for 24 to 48 hours. Lactic acid bacteria on MRS agar appeared as small, white to cream domed colonies, while spoilage microorganisms on PCA were generally yellow to cream varying in size from 1 to 5 mm in diameter. The catalase test was performed on PCA since lactic acid bacteria also grew slowly forming very small translucent or white, catalase negative colonies. These small colonies on PCA were not counted during enumeration. All values were expressed as $\log_{10}$ cycles. Microbiological methods used in this study were based on the procedure of Adams, Cooke and Twiddy (1987). However, this procedure was modified by using the standard pour plate enumeration method instead of the Miles-Misra technique.

Methods of Chemical Analyses

pH and total titratable analyses. Ten-gram samples were removed from each fermentation package and combined with 10 ml distilled water. pH was determined with a Corning Model 3D pH meter. The homogenate used in determining the pH was titrated against 0.1 N sodium hydroxide to pH 8.0. The titre volume was recorded and the %TTA calculated. This assumes that all acid present in the sample is lactic acid (Adams, Cooke and Twiddy, (1987).

Salt content. The Volhard Method (AOAC, 1975) was used to determine the salt content of the finished products.

Proximate analyses. Moisture, ash, crude protein, crude fibre, and crude fat were determined on the finished products using standard AOAC methods of analyses (AOAC, 1975).

Sensory Evaluation

"Burong tilapia" samples prepared traditionally and samples prepared with starters, coming from a single run and fermented for 7 days, were subjected to a 20-member panel and evaluated for odour, flavour, texture and general acceptability. The sauteed "burong tilapia" samples were evaluated using a five-point scale of quality-scoring.

Statistical Analyses

Microbiological counts, pH and total titratable acidity. The 2-factor factorial in a randomized complete block design (RCBD) was used in the experiment. The data obtained from microbial counts, pH, and total titratable acidity were subjected to the analysis of variance (ANOVA) (Snedecor and Cochran, 1967). Differences among treatment means were determined using the Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

Proximate, salt, and sensory analyses. The proximate, salt and sensory analyses data were analyzed using the ANOVA in RCBD. Differences among treatment means were determined using the DMRT.

RESULTS AND DISCUSSION

Two tables for count ratio, pH and total titratable acidity are given to show effects of fermentation days and starters.

Tables 1a and 1b show that the addition of commercial lactic starters significantly increased the initial population of lactic acid bacteria in the samples. The lactics are highly competitive against other microorganisms especially those responsible for spoilage. After 24 hours, it was observed that maximum MRS count had been reached since no significant differences were found from day 1 to day 7 for all treatments.

The key factor that favours a rapid rate of fermentation is the rapid proliferation of lactic acid bacteria with a concomitant decrease in the counts of spoilage microorganisms (Ames, 1987). This is determined by computing the log MRS minus the log PCA. It is advantageous to have higher MRS/PCA ratios. Preferably the MRS count should exceed the PCA count by 1 log cycle. Tables 2a and 2b show that the MRS/PCA ratio for samples with starters were significantly higher than the control up to the third day. This revealed that right at the start of fermentation (day 0), lactic acid bacteria were predominant over the spoilers. It can be observed that it was only on day 4 that the MRS/PCA ratio of the control became comparable to that of the samples with starters. By that time the desired effect due to fermentation had been achieved thereby discouraging undesirable changes that may be brought about by growth of spoilers.

Tables 3a and 3b show that no significant difference in pH was observed for all samples at the start of fermentation (day 0). From day 2 to day 4, samples with starters had significantly lower pH values that the control. After only 24 hours, the pH of "burong tilapia" was at pH 4.5 and below which is indicative of very rapid fermentation. For the control the pH dropped to less than 4.5 only on the sixth day of fermentation. According to Anderson (1989), the ability of lactics to become the predominating microflora depends on the initial population, their growth and acid production. Use of starters in preparing "burong tilapia" produces a high initial population of desired lactics, thus rendering such lactics the predominating organisms. As a consequence, acid is produced from the conversion of starch to lactic acid via the Embden-Mayerhof pathway. The acid produced inhibits other microorganisms particularly those that bring about spoilage. Results also revealed that the pH was maintained below 4.5 throughout the seven-day period. This is important for preservation and shelf life of the product.

Tables 4a and 4b show the percent lactic acid produced by the different treatments. Results revealed that maximum percent lactic acid produced by the control was 0.88% and was reached on the fifth day. For samples with starters, significant increases were observed and final lactic acid production was 1.42% after the seventh day. It is possible that lactic acid content may still increase if incubation time is extended. The use of starters increases the amount of lactic acid formed in fermenting mixtures.

Table 5 shows the proximate composition of "burong tilapia" samples. Results revealed that the use of starters did not significantly change the composition of the fermented rice-fish samples compared to those prepared traditionally or without starters.

Salt added to the total blend was 3%; however, salt analysis revealed that only 1.61 to 2.05% was present in the fermented rice-fish samples (Table 6). A portion of the salt is lost during preparation through drainage.

Table 7 shows that no significant difference were observed in all the sensory attributes evaluated. Addition of starters increased lactic acid content and the rate of fermentation but did not bring about significant changes in flavour and texture.

CONCLUSION

Addition of commercial lactic starters resulted in accelerated fermentation with a consequent increase in lactic acid content. What normally took 7 days required only 3 to 4 days without significant adverse changes in flavour and texture when evaluated on the seventh day. Savings in time, energy and other resources may be realized due to accelerated fermentation with use of starters. It is recommended that further studies be undertaken to monitor changes in microflora, pH, and total titratable acidity on extended incubation after 7 days and evaluate their impact on product quality. Likewise a cost-benefit analysis should be drawn to determine practicality of starter addition.

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Table 1a Log MRS-count of "burong tilapia" as affected by starter cultures and length of fermentation

DAYS	TYPE OF STARTER			
	Lacto-start	Pedio-start	Lacto-Pedio	Control
0	6.71b	7.96a	7.76a	4.26c
1	9.00a	9.11a	9.24a	7.65b
2	9.35a	9.25a	9.42a	8.50b
3	9.55a	9.46a	9.59a	8.61b
4 n.s.	9.01	9.52	9.56	8.75
5	9.64a	9.54a	9.29a	8.22b
6	9.51a	9.49a	9.48a	8.44b
7 n.s.	9.58	9.51	9.48	8.18

Means within the row following a common letter are significantly different.
n.s.: No significant differences among treatment means

Table 1b Log MRS-count of "burong tilapia" as affected by starter cultures and length of fermentation

DAYS	TYPE OF STARTER			
	Lacto-start	Pedio-start	Lacto-Pedio	Control
0	6.71b	7.96b	7.76b	4.26b
1	9.00a	9.11a	9.24a	7.65a
2	9.35a	9.25a	9.42a	8.50a
3	9.55a	9.46a	9.50a	8.61a
4	9.01a	9.52a	9.56a	8.75a
5	9.64a	9.54a	9.29a	8.22a
6	9.51a	9.49a	9.48a	8.44a
7	9.58a	9.51a	9.48a	8.18a

Means within the column following a common letter are not significantly different.

Table 2a Log MRS/PCA ratio of "burong tilapia" as affected by starter cultures and length of fermentation

DAYS	TYPE OF STARTER CULTURE			
	Lacto-start	Pedio-start	Lacto-Pedio	Control
0	2.64a	3.67a	3.38a	-0.30b
1	3.87a	3.41a	3.45a	1.50b
2 n.s.	3.07	3.26	2.42a	2.11
3	4.48a	3.91a	2.96	1.76b
4 n.s.	3.23	4.55	4.06a	3.18
5 n.s.	4.60	4.15	3.84	2.51b
6 n.s.	3.94	3.55	3.23	3.22
7 n.s.	3.89	3.82	3.75	3.18

Means within the row with a common letter are not significantly different at P >0.05

Table 2b Log MRS/PCA ratio of "burong tilapia" as affected by starter culture and length of fermentation

DAYS	TYPE OF STARTER CULTURE			
	Lacto-start	Pedio-start	Lacto-Pedio	Control
0	2.64c	3.67	3.38	-0.30c
1	3.87ab	3.41	3.45	1.50b
2	3.07bc	3.26	2.96	2.11ab
3	4.48a	3.91	4.06	1.76ab
4	3.23bc	4.55	3.84	3.18a
5	4.60a	4.15	3.23	2.51
6	3.94ab	3.55	3.62	3.22a
7	3.89ab	3.82	3.75	3.18a

Means within the column within a common letter are not significantly different at P >0.05

Table 3a The pH "burong tilapia" as affected by starter cultures and length of fermentation

DAYS	TYPE OF STARTER CULTURE			
	Lacto-start	Pedio-start	Lacto-Pedio	Control
0	6.03	5.96	5.88a	6.00
1	4.50b	4.40b	4.33b	5.48a
2	3.88b	3.90b	3.83b	5.00a
3	3.88b	3.50b	3.40b	5.00a
4	4.25b	4.05b	4.05b	4.75a
5 n.s.	4.20	4.20	4.20	4.60
6 n.s.	3.95	4.13	4.13	3.67
7 n.s.	4.00	4.15	4.10	3.88

Means within a row with a common letter are not significantly different at P >0.05

Table 3b The pH of "burong tilapia" as affected by starter cultures and length of fermentation

DAYS	TYPE OF STARTER CULTURE			
	Lacto-start	Pedio-start	Lacto-Pedio	Control
0	6.03a	5.95a	5.88a	6.00a
1	4.50b	4.40b	4.33b	5.48b
2	3.88e	3.90bc	3.83bc	5.00c
3	3.88e	3.50c	3.40c	5.00c
4	4.25c	4.05b	4.05b	4.75d
5	4.20cd	4.20b	4.20b	4.60e
6	3.95de	4.13b	4.13b	3.67g
7	4.00cde	4.15b	4.10b	3.88f

Means within a column with a common letter are not significantly different at P >0.05

Table 5 Proximate composition of "burong tilapia"

TREATMENT	PROXIMATE COMPOSITION					
	moisture (%)	ash (%)	crude protein (%)	crude fat (%)	crude fibre (%)	NFE (%)
Lacto-start	67.73	2.25	7.52	0.32	0.21	21.97
Pedio-start	67.41	2.16	7.97	0.90	0.27	21.29
Lacto-Pedio	68.39	2.58	9.49	1.66	0.40	17.48
Control (no starter)	68.14	3.12	7.54	0.12	0.58	20.55

Table 6 Salt content of "burong tilapia"

TREATMENT	% SALT
Lacto-start	1.61
Pedio-start	1.93
Lacto-Pedio	2.21
Control (No starter)	2.05

Table 7 Mean sensory scores of fermented "burong tilapia"

TRAITS	COMMERCIAL LACTIC STARTERS			
	Lacto-start	Pedio-start	Lacto-Pedio	Control
Odour n.s.	4.30	4.00	4.25	4.00
Texture n.s.	3.95	3.50	3.90	3.70
Flavour n.s.	3.75	3.20	3.45	3.35
General acceptability n.s.	3.80	3.35	3.55	3.30

n.s.: Treatments are not significantly different at P>0.05

CHANGES IN MICROBIOLOGICAL AND CHEMICAL COMPOSITION DURING FERMENTATION OF FERMENTED FISH (*PEDA*)

by

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ABSTRACT

Changes in microbiological and chemical composition in fermented fish (*peda*) during fermentation were investigated. It was found that total plate count increased for the first three days to 2.9 x 10^5 and then decreased until 15 days of fermentation. Free fatty acid and free amino acid contents constantly increased for 15 days to 20.10 g/100 g and 111.30 mg/100 g respectively. Histamine content increased and reached a maximum of 37.5 mg/100 g the ninth day, then decreased significantly during the remaining days.

INTRODUCTION

Peda is a traditional fish product made by salting followed by fermentation. Fermented fish, particularly *peda*, in Indonesia, is generally accepted by people because of their taste and flavour (Somaatmadja, 1983).

Fermentation is a preservation method of food, which is different from other methods, since in general the purpose of preservation of food is to decrease the number of bacteria. Fermentation needs the growth and reproduction of bacteria and also increases their metabolism (Winaro *et al.*, 1981).

Salt is the main component of fermentation of *peda*. Chemical compounds released by bacteria during fermentation also have an important effect on chemical composition. Therefore, it is necessary to study the microbiological and chemical changes in *peda* during fermentation.

This investigation was conducted by determining the total plate count (TPC), free fatty acid (FFA), free amino acid (FAA) and histamine contents during fermentation.

MATERIAL AND METHODS

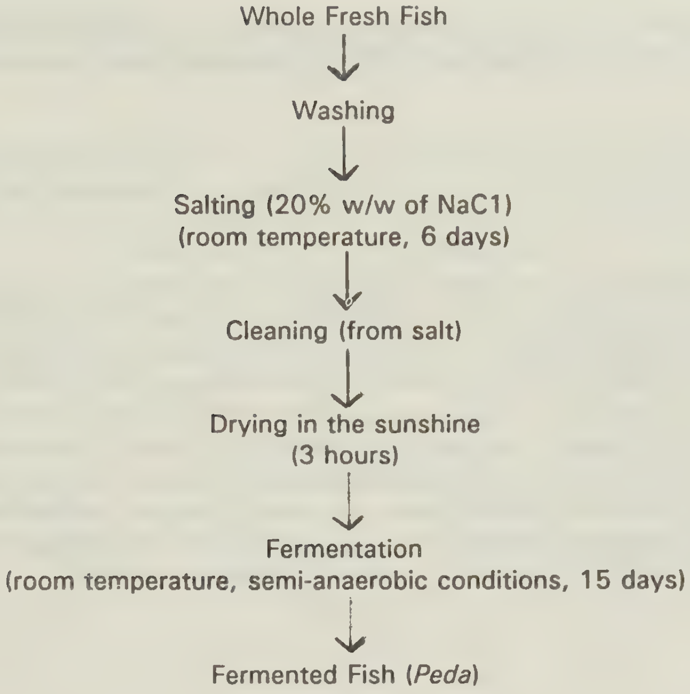
Processing of fermented fish (*Peda*)

The fish used was mackerel (*Rastrelliger brachysoma*) obtained from Jakarta Fishing Port. The fish was processed at NCQC using normal methods of Indonesian processors as shown in Figure 1.

Whole mackerel was salted by covering with food grade salt (20% of total weight of fish) for 6 days. Then the fish was cleaned of salt and dried in the sunshine for 3 hours. Fermentation was carried out by arranging the fish in a fermentation box under semi-anaerobic condition. Salt (10%of total weight of fish) was added and covered with dried banana leaf before fermentation was started.

The product was analyzed after 0, 3, 6, 9, 12 and 15 days while fermenting.

Figure 1. Flow chart of fermented fish *(peda)* processing



Analytical Methods

Determination of total plate count was undertaken according to AOAC (1984a). The sample was diluted with Butter Field Phosphate Buffer (BFP) solution and treated with a stomacher. Dilutions of the sample were transferred to petri dish with plate count agar (PCA), incubated for 48 ± 2 hours at 35°C. After incubation, colony counting used a colony counter and tally register.

Free fatty acid (FFA) content was determined according to Chemical Methods of Fisheries and Oceans Canada Fish Inspection Branch (1979). Percentage of FFA was calculated as oleic acid (% FFA = 0,5 x acid value). The oil in the sample was extracted by a mixture of chloroform and methanol. The oil was dissolved in benzene-ethanol (1 + 1) and titrated with potassium hydroxide using bromthymol blue as indicator.

The method of determination of free amino acid was taken from a 1938 paper of Pope and Steven (1938), used by RIFT in JKakarta as follows: Sample was extracted with 7% trichloroacetic-acid (TCA) solution. It was neutralized with 1 N sodium hydroxide solution using thymolphthalein as indicator. Cuppriphosphate suspension was added and filtered using filter paper. To the filtrate glacial acetic acid and 20% potassium iodide solution were added. Titration was done using sodium thiosulphate and starch as indicator and free amino acid calculated as amino N.

Determination of histamine was carried out according to Association of Official Analytical Chemists (AOAC, 1984b). Sample was extracted with methanol, then 20% sodium hydroxide solution and a drop of benzaldehyde added, and extracted with a mixture of benzene-butanol (3 + 2) by shaking. The mixture was passed through a CAS column. Histamine was eluted from the CAS with 0.4 N sulphuric acid solution followed by distilled water. The eluate was cooled in an ice bath, diazonium reagent, coupling buffer and disodium tetraborate powder added, then extracted to isobutile methylketone and washed with barbitol buffer. Absorbency was detected by Perkin Elmer UV - VIS spectrophotometer at 475 nm.

RESULTS AND DISCUSSION

The results of this investigation are presented in Table 1. The total plate count increased rapidly for the first 3 days, then decreased until the last day of processing as shown in Figure 2. This was possibly due to availability of nutrients in the medium to bacteria during the initial time of fermentation.

Increase of bacterial numbers occurred as a result. After 3 days, they decreased gradually, indicating that some bacteria had died, probably because of competition with other microorganisms. This was probably also influenced by their metabolic products.

Free fatty acid contents increased significantly after 3 days as shown in Figure 3. This agrees with those found by Sabina (1988) and Chang *et al.* (1991) who also found a marked increase of free amino acids.

Simple compounds such as free amino acid and free fatty acids possibly play an important role in the production of acceptable flavour characteristics of *peda* as mentioned by Chang *et al.* (1991) for fermented sardine.

Histamine content increased significantly until the ninth day of fermentation. However, it decreased significantly after than, as shown in Figure 5. Histamine is formed by bacteria which possess histidine decarboxylase through decarboxylation. Investigation of histidine decarboxylase bacteria in fermented mackerel (*R. neglectus*) done at NCQC laboratory showed *Morganella sp, Proteus sp, Klebsiella sp.* and *Hafnia sp.* (Prandaka *et al.* 1991). Chan *et al.* (1991) found the same trend of changes in histamine during fermentation in their report. Decreasing histamine after 9 days of fermentation was probably due to breakdown of the histamine or possibly because of reaction between the amine group of the histamine and the carbonyl group of the lipid oxidation product so that it was not detected by this analytical method. However, the accumulated amount of histamine was less than levels of histamine known to cause food poisoning (less than 50 mg/100 g, US FDA Standard) (Ababouch, 1991).

CONCLUSION

Changes in the total plate count, free fatty acids, free amino acids, and histamine contents, occurred during fermentation. Total plate count and histamine contents increased initially and then decreased. After 15 days of fermentation the free fatty acid produced was 20.19 g/100 g and the free amino acid was 111.30 mg/100 g.

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Table 1. The changes of TPC, FFA, FFA and Histamine of Fermented Fish during Fermentation

T (days)	TPC (log number)	FFA (g/100 g)	FAA (mg/100 g)	Histamine (mg/100 g)
0	3,51	11.48	91.88	13.26
3	5.46	11/65	103.43	18.35
6	4.45	13.95	106.05	30.65
9	4.36	16.48	109.02	37.45
12	4.39	18.02	110.25	14.48
15	2.34	20.19	111.30	6.18

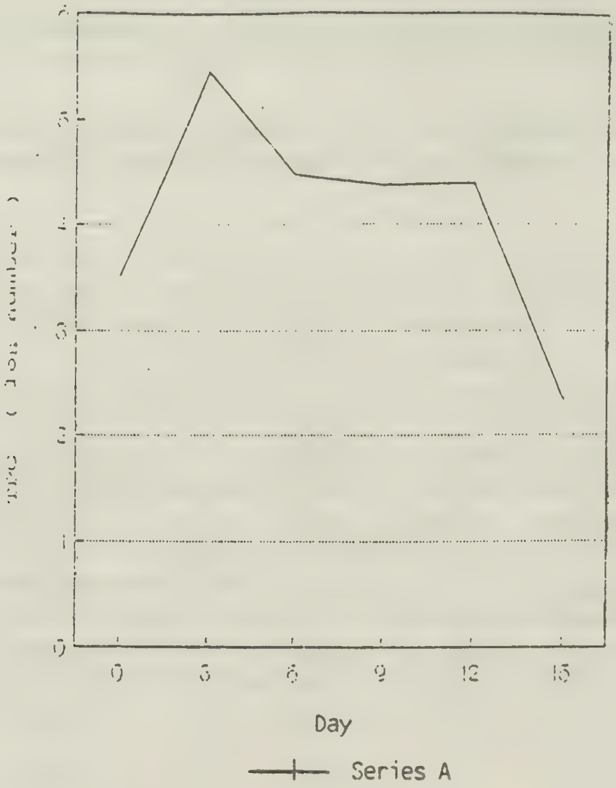

Figure 2 Changes of TPO during fermentation

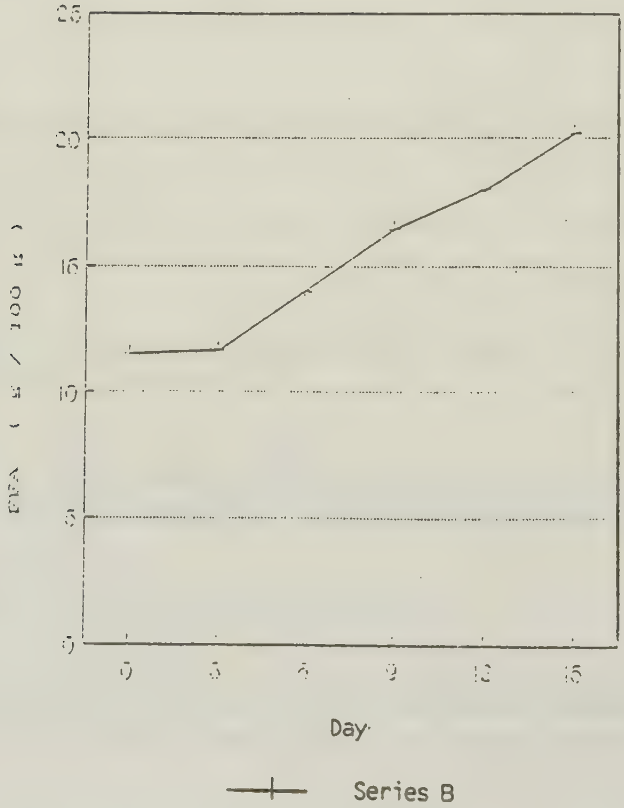

Figure 3. Changes of FFA during fermentation

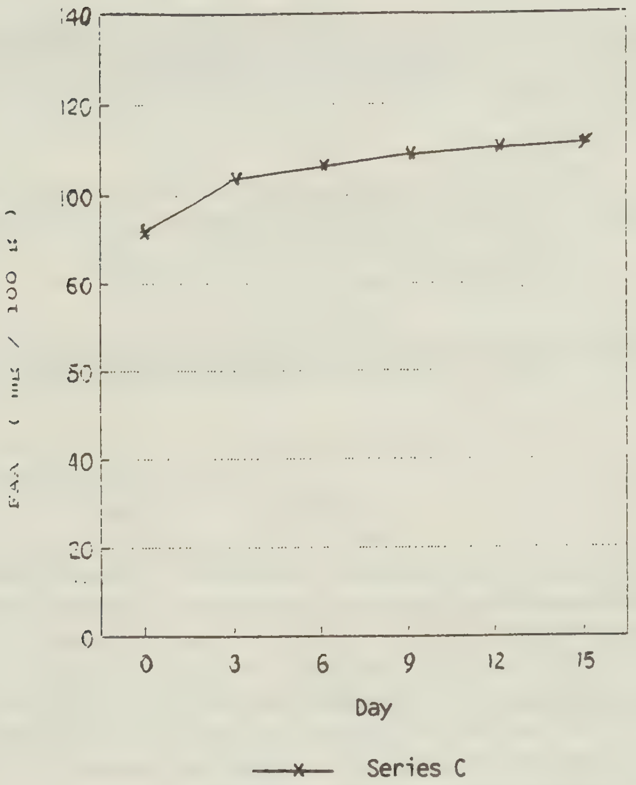

Figure 3 Changes of FAA during fermentation

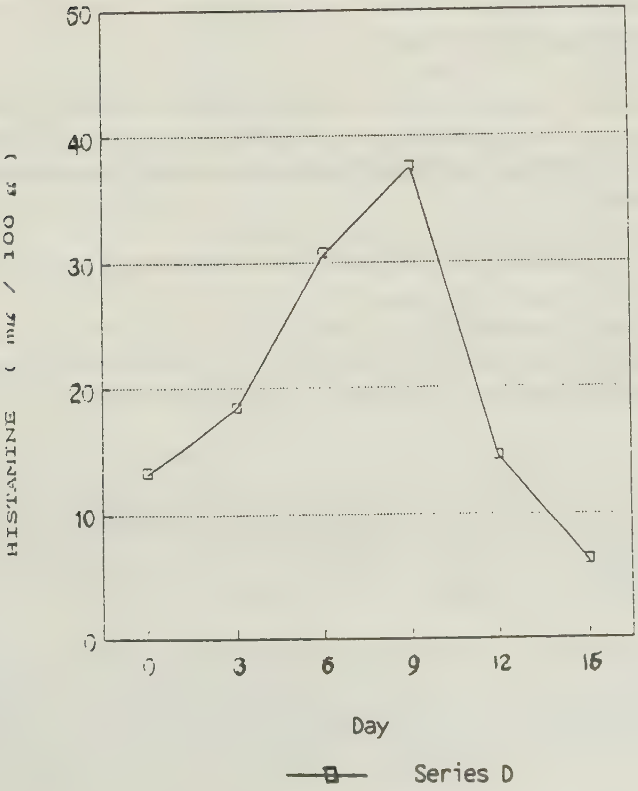

Figure 4. Changes in Histamine during fermentation

ENSILING OF FISH BY LACTIC ACID BACTERIAL FERMENTATION FOR FEED PRODUCTION

by

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ABSTRACT

The paper discusses the possible application of lactic acid bacterial fermented feed, as a technology to increase the utilization of fish raw material and fish waste.

Discussion is focused on the following topics:

1) Comparison with the acid silage technology.
2) Bacterial lactic acid fermentation.
3) Probiotic concept.
4) commercial considerations,
5) R & D and technology transfer.

UTILIZATION OF FISH WASTE IN FEED

Fish technologists have for decades been occupied with the overall aim to improve and upgrade the utilization of fish raw material. This applies also to so-called fish waste, that either is not utilized or where improvements in technology will result in better application of this raw material.

Fish waste and/or so-called trash fish can be used:

1) raw or cooked, in animal or fish feed
2) as fish meal, in animal or fish feed
3) as fish silage, in animal or fish feed
4) as fermented fish, in animal or fish feed
5) as fertilizer.

Previously, feed products made from fish raw material were mainly used as animal feed. However, with the introduction of fish and shrimp aquaculture, fish feed products have increasingly been used in the feeding of these species. Thus, in Norway, with the increased production of salmon, all of the fish meal produced is now used in salmon feed, and the industry is looking for additional fish resources to be used as fish feed.

The Norwegian fishing industry is traditionally producing salted and dried products, frozen fillets, peeled shrimp, etc. The production results in by-products that are often classified as waste, e.g.

1) fish viscera
2) fish heads
3) fish trimmings
4) shrimp shell.

Some of this waste is raw material in fish meal production and/or used as frozen feed for fur animals and fish. However, 50-100,000 tons of this raw material is wasted annually (Landfald and Lovland, 1990).

In the mid 70's work was started in Norway to improve the utilization of fish waste, using the acid silage technology. This coincided also with international interests in this field (Disney and James, (1980).

FISH SILAGE

In a paper by Raa *et. al.* (1983), the theory and practice of fish silage are reviewed. The main factors contributing to a good fish silage are:

1) Fresh raw material,
2) Cut or chop raw material to pieces,
3) Add acid to pH 4 or below,
4) Preferably use organic acids; e.g. formic acid.

An important factor in silage preparation and subsequent storage is the liquefaction (autolysis) of the fish material due to the action of enzymes (pepsins). This autolysis may represent a problem for its use. Another factor is the growth of mould, which may be controlled by fungicidal acids like propionic and sorbic acid. Most fish species contain thiaminases which degrade vitamin B. This problem and the possible problems of autolysis can, however, be controlled by heating the silage, thereby inactivating these and other enzymes. Heating of silage is also a safeguard against the spreading of possible acid resistant virus, and heating makes oil removal from material with high lipid content easier.

In Norway, approximately 40-50,000 tons of silage was produced in 1990 (Stormo, 1991). Very little of the silage is used as standard fish silage (dry matter of 15-18%), the main reasons being lack of standardization and quality control of the silage (Jahnsen and Landfald, 1990). The bulk of the silage is instead produced as a silage concentrate (d.m. ◇-45%), in an industrial process described by Raa *et. al.* (1983). The silage concentrate is subsequently used in dry feed pellet productions, by adding approximately 5% of the silage concentrate to the other feed ingredients prior to pelleting (Stormo, 1991). This use of silage was tested out in 1976 (Stormo and Strøm, 1977). However, it is only recently that silage is used industrially by this technology.

Internationally, there has not been similar developments regarding the industrial use of fish silage, and as far as the authors are informed, silage technology is not applied industrially in S.E. Asia.

PRESERVATION BY LACTIC ACID BACTERIAL FERMENTATION

Lactic acid bacterial fermentation is extensively used in the production of milk products for human consumption and in preservation of feed, e.g. hay, corn (for review see Peppler (1983). This technology has also been tried with fish raw material (Raa, et. al. 1983), however, without any commercial success.

The fermentation of fish by lactic acid bacteria is characterized by:

1) Low initial numbers of lactic acid bacteria in fish; 101-104/g
2) Low concentrations of sugars (carbohydrates), which is essential for the growth of lactic acid bacteria.

Thus, both a starter culture and a carbohydrate source is required as additives for a successful fermentation of fish raw material.

In the review by Raa *et. al.* (1983) references are given to reports describing fermentation of fish raw material. We have summarized some of these findings below:

1) Carbohydrate source; e.g. 20% malt + oat meal, 10% molasses.
2) Starter culture not specifically required, but recommended.
3) pH lowered to approximately pH 4.

4) At pH 4, spoiling bacteria and pathogens are destroyed (e.g. coliforms, enterococci, typhoid bacteria, and even spores of *Clostridium sp.).*
5) Development of ammonia; may be controlled by combining acidulation and sugar addition.
6) Tropical waste fish can be stored for at least I month.
7) Fermented fish maintains a good nutritional value during longterm storage.
8) Rancidity is prevented even during drying.

During fermentation, the lactic acid bacteria produces lactic acid, which acts as a food and feed preservative. In addition, these bacteria produce other components which also inhibit spoilage, as reviewed by Gibbs (1986). The inhibitory components produced by lactic acid bacteria are:

1) Lactic acid
2) Volatile acids
3) Hydrogen peroxide
4) Antibiotic-like compounds

From these results and other findings (e.g. Bastien, 1990, with animal offal), it seems that the production of fermented feed products is technically possible. However, the question whether fermentation of fish raw material used as feed is a good commercial alternative, remains to be answered.

In Norway, the company Biotec-Mackzymal, Tromsø, has carried out pilot plant production of a special fermented product, named EnsiFerm. The production of a stable product was carried out by:

1) acidulation,
2) heat treatment; pasteurization and inactivation of enzymes,
3) addition of starter culture,
4) addition of carbohydrate (10%),
5) fermentation at 30-40°C,

giving a fermented product with pH 4.1-4.3, TVN <70 mgN/100g, histamine <1 mg/kg, putrescine <20 mg/kg and cadaverine <50 mg/kg.

This process is not commercialized.

FERMENTED FISH AS FEED; PROBIOTIC CONCEPT

The use of fermented feed products in animal feeding, e.g. pigs and poultry, has attracted the interest of both scientists and commercial companies. Of special interest has been the positive effect of such feed on problems concerning disease, (e.g. diarrhoea). There are several factors which seem to contribute to the positive effect of fermented feed products. Those that contribute to an improved health situation for the animal are called "probiotics", which are controlling factors of the intestinal flora of the animals. The term "probiotic" is attributed to Parker (1974), who defined them as "organisms and substances which contribute to intestinal microbial balance". For review see Sissons (1989).

Microbial flora of intestines

The microbial flora of intestines is complicated. A so-called normal flora consists of many different organisms in number as high as 10^{12} bact./ml (Goldin, 1986).

During feeding, this flora can be manipulated by varying the composition of the feed. Thus, in feed formulation it is important to introduce factors which can control the growth of bacteria that e.g. result in diarrhoea. In addition to controlling the nutrients used in the feed, research has shown, for instance, that unwanted coliform can be controlled by feeding lactobacilli to the animals (Sissons, 1989).

Probiotic factors

The mode of action of lactic acid bacteria is attributed to several factors as summarized below (Sissons, 1989):

1) competitive attachment,
2) bactericidal activity,
3) enterotoxin neutralization,
4) prevention of toxic amine synthesis,
5) enhanced immunity.

In addition to preventing diarrhoea, it is interesting to point out that lactic acid bacteria, by their synthesis of antibiotic-like components (e.g. Muriana and Klaenhammer, 1991), may result in improved growth as a result of the so-called antibiotic growth effect (Jukes, 1978). This growth effect may be caused by the prevention of growth of bacteria that are synthesizing enzymes which catalyze the hydrolysis of bile salt, (Feighuer and Daslikevicz, 1986). Such hydrolysis will result in reduced ability to digest lipids.

The observation that intraperitoneal injection with lactobacilli raised the activities of macrophages and lymphocytes in mice, indicates a role of lactobacilli in the unspecific immune system of animals (Sissons, 1989).

Fermented fish as feed to fish

There is little information regarding fermented fish as feed to fish. All the work referred to above is with warm blooded animals. Some work has been carried out on the controlling effect on diarrhoea in fish by lactic acid, used as preservative/additive in the feed (Ringo, 1991). Regarding the other probiotic factors, research is called for in this very fascinating field.

ECONOMICS

The cost of producing silage in Norway is given below (Stormo, 1991):

Silage production:

Raw material		100 NOK/ton
Silaging cost		250 NOK/ton
Overheads, etc.		150 NOK/ton
	Total	500 NOK/ton (80 US$/ton)

If this silage is used for producing silage concentrate (45% d.m.), using ~3x the amount of silage, the costs are:

Silage concentrate production:

Raw material		1500 NOK/ton
Defatting, concentration		550 NOK/ton
Overheads, etc.		950 NOK/ton
	Total:	3000 NOK/ton (480 US$/ton)

The costs of producing fermented feed is less certain:

Fermented fish production:

Raw material	100 NOK/ton
Starter culture	100 NOK/ton
Carbohydrate	100 NOK/ton
Production cost	?
Investment cost	1 - 2 mill US$/ton (?)

It is obvious from the above that more information is needed, both from research and from industrial studies, to present a firm base for the possible introduction of this technology in the fishing industry and in aquaculture.

FUTURE WORK

In our opinion, lactic acid bacteria fermentation of fish and fish waste is a technology for the near future, with applications in animal and fish feeding both in tropical countries as well as in Norway.

However, the required R & D and the task of technology transfer to the industry must be organized so that the industry is presented with results from R & D as well as pilot production and field trials.

Our experiences from development of the silage technology in Norway are summarized below:

1) The silage technology is based on extensive basic and applied R&D.
2) Pilot production and field trials were carried out in cooperation with the industry.
3) It has taken 10-15 years from the start of the silage project to its successful commercial application.

On the basis of this experience we will make the following recommendations for the further development of the fermented fish technology:

1) R & D should be carried out in areas of:

   - Fermentation of fish with lactic acid bacteria
   - Nutritional value of fermented feed
   - Health in fish and animals fed on fermented fish

2) Pilot plant studies
   - Large scale fermentations
   - Feeding trials
   - Economics of fermented fish

3) Commercial trials
   - Long term practical feeding trials
   - Overall economics

4) Logistics
   - Small scale applications
   - Large scale industrial applications

The R & D should be organized in joint R & D programmes with the parties involved, preferably within the IPFC working party on fish technology.

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EVALUATION OF FISH SILAGE AS PROTEIN SOURCE FOR POULTRY

by

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ABSTRACT

Two samples of fermented fish silage (fish, sargassum, molasses mixture, Lot A, and fish, molasses mixture, Lot B) were evaluated as feed supplement products. The dried and ground fish silage products were used as major protein sources in prepared poultry feeds and were assessed for their nutritional quality in a feeding study done with broiler chicks for six weeks. The control rations (commercial feed) gave satisfactory weight gain, good appetite and healthy chickens with thick plumage. The feeds containing silage gave a lower weight gain, less appetite and chickens with leg weaknesses and thinner plumage. The feed conversion efficiency value were however comparable with those of the control diets. Economics of the rations are presented.

INTRODUCTION

Poultry raising in the Philippines is a popular source of income both in the rural and suburban communities. Producers, however, still rely on the commercially produced feed-stuffs although these are quite expensive. Small-scale producers normally modify the diets to save on feed expenses.

In recent years, the profitability of using non-conventional feeds as a protein source or as a feed supplement for poultry has gained considerable attention. Fish silage preparations as poultry feed have been studied intensively in Asia and the results obtained have been promising. Feeding experiments were done in the Philippines, however, results were not as good as in other countries. The nutritive values were quite good although not very well established.

Fish silage as a cheaper substitute, wholly or in part, for the protein source has generally shown fluctuation in the trend of growth of poultry. This study was undertaken to compare previous local feeding results using fish silage and to determine its potential as a substitute for expensive protein sources. It aimed to evaluate the nutritive value of two lots of fermented fish silage: fish- sargassum- molasses and a fish-molasses mixture, for poultry, based on proximate analysis of the silage, its efficiency as feed, and the growth response of poultry when protein is supplied by fish silage alone.

MATERIALS AND METHODS

Fish Silage Production

The flow diagram which summarizes the procedure used for the experiment is shown in Figs. 1 & 2, assorted marine fresh fish of low commercial value, i.e. dansel fish, boxfish and lizardfish, were ground and divided into 2 lots of 15 kg each. Similarly, dried sargassum was ground and weighed at 15% of the ground fish, then mixed thoroughly (Lot A). Molasses was added to this lot at 25% by weight of the mixture. The second lot, Lot B, was mixed with molasses alone at 25% by weight of the ground fish. Both mixtures were thoroughly mixed with a wooden paddle and kept in tightly closed containers at room temperature (24-28°C) for 15 days. Stirring was done daily. Samples of the mixtures were withdrawn on a 3-day interval i.e. 0, 3, 5, 7, 9, 12, and 15, for chemical analyses: amino-N, titratable acidity, reducing sugars and pH. The analyses were all based on standard procedures. The products were sun-dried to concentrate the protein content, and then pulverized to a fine powder. Samples of the dried pulverized products were taken for proximate analysis using standard methods.

Experimental Diets

The diets were made isoproteinic using the Pearson's square formula. Feed components such as complete vitamin and mineral premix and oyster shell meal were mixed thoroughly. The proportions are shown in Table 1. Commercial diets (chick starter, growing mash, and broiler finisher) were used as control diets. These were obtained from poultry supply stores. A proportion of each ration was taken for proximate analysis.

Experimental set-up

The chickens were divided into three cages, each cage containing 12 chickens. Acclimated with chicken booster feeds for three days. They were then fed with Feed 1, Feed 2, and the control. Drinking water with added antibiotic was supplied abundantly and changed daily, for the first 15 days. Prior to the start of the feeding experiment, the chickens weighed an average of 61.67g. They were fed ad libitum, i.e. when the feeding tray was empty, additional feed was given. Weight gain and feed intake were recorded weekly for six weeks. Feeds were assessed based on feed conversion ration (FCR)[1] and feed conversion efficiency (FCE)[2].

RESULTS AND DISCUSSION

Fish silage products

Both liquid products (Lot A & Lot B) exhibited physical changes during ensiling. The odour, colour, consistency and mould colonies were checked every day. It was observed that both silage preparations displayed a slightly ammoniacal to sweet odour as days of ensiling progresses. Lot B appeared dark reddish brown while Lot A was dark brown. Lot A held less water present in the fish.

The presence of sargassum made Lot A more difficult to stir than Lot B. Fibrous components of this ingredient slowed down hydrolysis in Lot A which could account for more effort applied in stirring the mixture thoroughly.

The results of the chemical analyses of the two lots are given in Table 2. Amino -N content of both lots increased with time (Figure 3) but higher values were obtained in Lot B. This indicated a higher rate of hydrolysis occurring in Lot B, which resulted in the formation of more free amino acids. Lot A was observed to have lower values because sargassum could have delayed the hydrolysis (Huit 1986).

Similarly, titratable acidity increased with time (Figure 4). Carbohydrate conversion to lactic acid was more rapid in Lot B than in Lot A due to the bacteria which are in direct contact with the sugars. The sargassum in Lot A could have buffered the carbohydrate conversion to lactic acid. Available glucose in the two lots showed opposite trends (Figure 5). In Lot A, sugars were made available gradually until the 15th day. Huit (1986) theorised that the complex carbohydrates present in sargassum had adsorbed some sugars and prevented their initial release. Gradual fragmentation of the complex carbohydrate allowed the later release of the reducing sugars. Consequently, the absence of such a complex molecule in Lot B favoured rapid release of the sugars early during ensilage thereby decreasing availability on the 15th day. The drop per sampling day, though, was gradual. A drop in pH was great on the third day for Lot B, then remained fairly constant at 4.6 until the 15th day (Figure 6). Constant pH was obtained on the 9th day for Lot A. This difference is accounted for by a faster rate of hydrolysis in Lot B thereby providing a more acidic environment than Lot A.

Though the pH became fairly constant early during the ensiling period, titratable acidity still increased. The pH of the systems should have decreased further but salts present abundantly in the fish prevented such occurrence by acting as a buffer (Huit, 1986). It is also possible that the presence of sugar in the molasses hindered the production of dominating enzymes in bacteria, hence a decrease in pH. Raa & Gildberg (1984) stated that the growth of acid tolerant lactic acid bacteria which produce sufficient acid to maintain the low pH is favoured by the presence of sugar.

After ensiling, the two lots were subjected to sundrying. Lot A, dried after three days, while it took seven days to dry Lot B. Initially and even during ensilage, Lot A appeared less of a liquid and contained solids, while Lot B, was viscous. Problems were encountered with Lot B during grinding because it readily absorbed moisture from the atmosphere and therefore became sticky. The sample was left under the sun even during the duration of grinding to prevent possible moisture reabsorption.

Table 3 gives the proximate analysis results of the dried ground silage preparations. The percentage crude protein and crude fat were higher in Lot B than in Lot A, the crude protein obtained was 27.85% and 38.40 % respectively. The presence of sargassum in Lot A could have influenced the total crude protein by offsetting further nitrogen release. Extraction of more fat was possibly blocked by the same component, thus giving lower crude fat percentage in the same lot. The moisture content was high in both silage samples which required a need for dry components to absorb water. Ash content differed greatly where Lot B's value is indicative of more minerals present in the dry matter. The fibre content in Lot A as expected is higher than in Lot B because of the fibrous nature of the seaweed component.

Feeding study results

The percentage composition of the diets are given in Table 4 and the results of the feeding trial using the rations are summarized in Table 5. There was a significant difference in weight gain between poultry groups fed with Feed 1 and the control. The same trend was observed between Feed 2 and the control. Neither Feed 1 or 2 exhibited much weight gain.

This result is significantly different from the control's effect on chicken growth as shown by higher weight gain. Animals given the control diets fed more aggressively (feed intake/chick = 1483.45 g) than animals given either, Feeds 1 or 2 (feed intake/chick = 1156.89 and 1092.56 g respectively). Such a difference in appetite could be traced from the general appeal of the feeds to chicks. Diets which contained silage were more powder and dark-coloured, more sticky when taken and easily absorbed moisture. The control diets with varied particulate components size, were light coloured and dry. Although poultry are generally known to be nonselective with feedstuffs, the animals in the study discriminated against the trial rations as observed by a decrease in appetite when fed. Chickens uniformly exhibited good appetite when previously fed chick booster. Aside from the general appeal of the feeds, it was postulated that the amino acid profiles of the silage contained feeds, may be inferior to the control rations.

Feed conversion ratio for Feed 1 was higher than either Feed 2 or the control. This is indicative of an economical ration. Sargassum in the diet could have influenced feed efficiency by increasing the chicken's weight (Okazaki, 1971). Aside from providing food bulk, its high water holding capacity (due to its fibrous nature) helped maintain a moist and soft faecal mass (Schaible, 1970).

The poultry's weight gain per gram of feed given was not significantly different in the three diets ($P>0.01$). Droppings were less in silage-fed chickens and defecation occurred hours after feeding. Droppings from the control-fed chickens were more in bulk and defecation occurred shortly after feeding. This observation may imply an increased absorption of nutrients among chickens fed with diets incorporated fish silage than those in the control diets. An assay of the animal has to be done however to confirm this speculation.

Slow growth of feathers and leg weaknesses were evident in silage fed chickens. Although recommended levels of vitamin and mineral inclusion were followed, the conditions were not avoided. Poulter and Jayawardena (1979), observed similar results but corrected the conditions by giving excess vitamins and minerals.

Economics of the rations are given in Table 6. Feed expenses when compared showed that the fish silage diets are 40% cheaper than the control rations. It was not established however, whether fish silage or commercial feed production as a whole costs more, because no data are available for commercial feed preparation expenses.

SUMMARY AND RECOMMENDATIONS

Two lots of fermented fish silage were evaluated as products and as feed protein sources in poultry. Preservation of the product was easily made possible due to a rapid drop in pH. The rate of hydrolysis in both preparations increased with time. The same trend was observed when titratable acidity was determined. Available glucose decreased with ensiling time in Lot B because it was more susceptible to degradation of lactic acid by lactic acid bacteria. An opposite result was obtained for Lot A. The presence of sargassum in Lot A could account for higher available glucose during ensilage because the complex carbohydrate absorbed sugars first, then released them. Low titratable acidity values were obtained for this lot also because of the seaweed's buffering effect which caused a delay in the rapid drop of pH.

The highest weight gain was obtained from chickens fed with control rations. Although weight gain in chickens fed either Feed 1 or 2 was low, values for FCR and FCE were comparable with the control. Growth limiting amino acids could have caused stunting among silage fed broilers but as a whole, the strain of the animal might have played an equally vital role in growth response. Such trends for FCR and FCE coupled with better growth response would make silage diets a potential for cheaper poultry feed. Aggressive feeding was exhibited by the control-fed chickens. The silage diets were eaten with less appetite probably due to a lower general appeal to the chickens, although such an observation need to be further investigated. Leg weaknesses and slow feather growth were not avoided in silage-fed chickens.

The possibility of incorporating rice bran as an additional energy source needs to be explored. Rice bran counteracts moisture absorption better than corn meal but in other respects, corn provides more energy. The incorporation of rice bran in the diet may enhance the feed's general appeal to the chicken, owing to its lighter colour and drier nature. Levels of vitamin and mineral inclusion need to be established locally so that test animals do not suffer from rickets or perosis. Feed assessment can be more comprehensive by better mineral, vitamin and amino acid analysis of the rations. This phase was not made in the study due to lack of facilities in the laboratory.

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Table 1. Composition of experimental rations.

Ingredients	Days 1-15		Days 16-30		Days 31-45	
	Feed 1 (g)	Feed 2 (g)	Feed 1 (g)	Feed 2 (g)	Feed 1 (g)	Feed 2 (g)
Fish silage	63.35	56.75	38.76	25.10	40.00	31.75
Corn meal	34.15	42.75	60.74	74.40	50.50	67.75
Vitamineral mix (Afsillin)	0.25	0.25	0.25	0.25	0.25	0.25
Oyster shell meal*	0.25	0.25	0.25	0.25	0.25	0.25
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00

*recommended by the Bureau of Animal Industry (BAI)
Feed 1 - with Lot A silage
Feed 2 - with Lot B silage

Table 2. Results of the chemical analyses of liquid silage.

Day	mg amino-M per g sample		% T.A.		mg 5 reducing sugar (glucose)		pH	
	Lot: A	B	A	B	A	B	A	B
0	1.81	1.12	0.47	0.76	8.85	9.87	6.6	6.7
3	4.86	4.42	0.97	1.20	16.28	25.45	5.0	4.8
7	5.12	6.63	1.10	1.20	16.37	23.92	4.7	4.6
9	5.98	7.12	1.31	1.42	39.87	20.71	4.6	4.6
12	7.35	8.49	1.47	1.82	41.69	17.87	4.6	4.6
15	7.56	9.30	1.85	1.92	42.51	15.18	4.6	4.6

Table 3. Proximate composition of dried silage

Components (%)	Lot A	Lot B
Crude Protein	27.85	38.40
Crude Fat	6.64	8.52
Moisture	21.85	21.82
Ash	19.01	28.70
NFE and fibre component*	24.65	12.46
Total:	100.00	100.00

*NFE and fibre = 100.00 (sum of other components)

Table 4. Proximate composition of commercial and experimental diets

Components %	Days 1-15			Days 16 - 30			Days 31 - 45		
	Feed 1	Feed 2	Chick starter	Feed 1	Feed 2	Growing mash	Feed 1	Feed 2	Broiler finisher
Crude protein	21.49	22.21	21.47	16.68	16.25	17.16	18.54	18.45	18.42
Crude fat	5.12	3.86	2.92	2.81	2.47	3.57	4.04	3.56	3.40
Crude fibre	4.30	2.50	6.80	5.40	5.80	5.40	7.20	6.60	4.90
Moisture	16.19	14.09	12.01	17.12	15.28	12.73	15.53	13.68	13.21
Ash	14.91	8.61	12.76	8.57	4.48	7.82	9.34	7.14	6.44

Table 5. Summary results of feeding experiment rations and commercial feed to broilers

Feed	Average initial weight	Weekly Average Weight						Average final weight	Average weight gain	Average feed intake/chick	FRC	FCE
		1	2	3	4	5	6					
1	61.83g	65.45	80.44	129.33	208.56	327.70	473.20	473.20	407.75	1156.89g	2.83	0.35
2	59.76g	65.44	85.98	121.57	182.57	307.52	400.65	400.65	340.89	1092.56g	3.20	0.31
Control	63.42g	84.95	122.38	170.78	270.94	404.90	549.35	549.35	485.93	1483.45g	3.03	0.33

Feed 1 = with Lot A silage as protein source
Feed 2 = with Lot B silage as protein source
Control = commercial rations

PREPARATION OF FISH SILAGE BY MICROBIAL FERMENTATION

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ABSTRACT

Studies were carried out to prepare microbial silage using locally available low-cost trash fish and different carbohydrates. Out of many carbohydrate sources and their combinations tried, a mixture of sucrose and molasses (1:1) when used 10% (w/w) to fish, proved to be the cheapest for silage preparation. Merits of possible starter cultures were also evaluated.

Microbial and acid silage were stored at ambient temperatures (28°-30°C) and the changes during storage are mentioned. The production of TMA-N, VE-N and NH_3 in microbial silage were much higher, during storage, compared to acid silage. No noticeable difference was found in the extent of hydrolysis between the two silages. Other changes also occurred in a similar fashion in both the silages. However, the odour of microbial silage is more acceptable than acid silage throughout the storage.

INTRODUCTION

Microbial fish silage is the end product of controlled fermentation process in which carbohydrates are added to whole minced fish or fish waste and fermented by addition of lactic acid bacteria. Production of lactic acid or other organic acids result in lowering of pH. This reduction in pH is one of the primary causes of inhibition of spoilage and pathogenic organisms.

Considerable interest has developed in the preparation of fish silage by fermentation and preparation of fish fermented products during previous years (Roa, 1965; Burkholder *et al*, 1968; James *et al*, 1977; Stanton & Yeoh, 1977; Herborg & Johansen, 1977; Disney & James, 1980, Raa & Gildberg, 1982; Lindgren & Pleje, 1983; Adams *et al*, 1985; Adams *et al*, 1987; Cooke *et al* 1967; Karmas & Lamber, 1987; and Twiddy *et al*, 1987).

Studies on the preparation of acid fish silage, carried out in these laboratories with a view to introduce it as an alternative means of utilizing trash fish, have already been reported (Fatima & Qadri, 1987).

The purpose of studies reported here was to study the merits of sauerkraut as a starter inoculum, to prepare microbial silage under local conditions using local raw material and to compare and evaluate the merits of fermented silage over acid silage for a storage period of one year.

The maintenance of starter culture is, no doubt, a serious handicap at the village level (Disney *et al*, 1977). To solve this problem the merits of sauerkraut, to be used as starter inoculum, was also evaluated in comparison with the pure *Lactobacillus* culture.

MATERIALS AND METHODS

Trash fish (mainly, sardine) was selected for the study because *Sardinella sp*. form the bulk of trash fish and are found throughout the year. Samples were collected in clean plastic bags from trawlers landing at the fish harbour. Fish well surrounded with crushed ice, were brought to the laboratory within 1-2 hours of collection. After washing with tap water the fish were frozen overnight at -30°C before the start of the experiment.

In an attempt to select an appropriate fermentable carbohydrate to be used in the preparation of fermented silage the following preliminary studies were carried out. Different carbohydrates available locally, namely sucrose, liquid glucose and molasses at 10% level individually and 5% each in combination with 5% molasses i.e. 5% sucrose + 5% molasses, 5% liquid glucose + 5% molasses and 5% sucrose + 5% liquid glucose were tried. Two sets of these carbohydrates at 10% (w/w) level mixed with 200gm of minced fish, each were stored in plastic containers, one set of six containers was inoculated with broth culture of *Lactobacillus plantarum* and the other set with 10% w/w sauerkraut as starter inoculum for silage preparation at 28-30°C. Yeast and mould growth was inhibited by using propionic acid. Samples were taken out at 3, 7, 14, and 21 days for determining the changes in pH, non-protein nitrogen (NPN), volatile basic nitrogen (VB-N) trimethylamine nitrogen (TMA-M), ammonia (NH_3-N) and bacterial counts. Six such experiments were carried out and results were expressed as mean of six values. For preparation of fermented and acid silage 2 kg of fish were minced well in an electric meat mincer and divided into two parts. One part was mixed thoroughly with 5% (w/w) sucrose and 5% (w/w) commercial molasses and 10% (v/w) active culture of *Lactobacillus plantarum* grown in MRS broth. The slurry was thoroughly mixed, kept in covered plastic jars and allowed to ferment at ambient temperatures (28-30°C). To the other part, 3.5% (v/w) formic acid was mixed thoroughly in another plastic container and stored at the same temperature. After 48 hrs of storage the silages were stirred and sprayed with pure propionic acid (Riedel-deHain) to inhibit the yeast and mould growth.

For comparative quality evaluation, samples were drawn at suitable intervals to determine changes in different quality parameters (detailed in the preliminary studies) over a period of one year.

Preparation of Sauerkraut

Sauerkraut was prepared by mixing shredded cabbage with 2.5% NaCl and kept in a glass or plastic container at 28-30°C for 8 days. The pH of sauerkraut was found to be 4.0.

Isolation of *Lactobacillus plantarum*

The culture of *Lactobacillus plantarum* was isolated, from sauerkraut prepared in the laboratory, on MRS agar (Oxoid). The organism was identified according to the Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974). For preparing the inoculum a loop full of culture from MRS agar slant, was transferred to 10 ml of MRS broth (Oxoid) and incubated at 30°C for 24 hrs.

Plating Procedure

Lactobacillus counts were determined by the spread plate method, placing 0.1ml of appropriate dilution of silages on MRS agar. The plates were incubated at 30°C for 48 hrs.

Analytical Procedures

The analyses of NPN (soluble nitrogen), VB-N, TMA-N were performed on 10% trichloroacetic acid (TCA) extract of fish silage samples by 1-2 minutes shaking with a sample to solvent ratio of 1:3.

Protein (Nx6.25), were determined according to AOAC procedures (AOAC, 1975).

TMA-N was estimated by Dyer's picric acid procedure (1945) as modified by Hoogland (1956).

VB-N was determined according to Cobb *et al* (1973). NH_3 was obtained by subtracting TMA-N from VB-N. For the measurement of pH, a slurry of silage was used and pH was measured by using a Titri pH meter (Radelkis).

RESULTS AND DISCUSSION

The Figures 1 and 2 present the changes in pH, TMA, VB, and bacterial counts of silages made from different carbohydrate sources with the inoculation of *L.plantarum* or sauerkraut as starter, respectively.

No difference was noticeable between fish silage samples prepared using different carbohydrate sources and different types of inocula during 21 days of storage. All samples attained a pH between 4.5-4.6 within 3 days and remained almost unchanged throughout storage. The pH of all silages after 21 days was between 4.5-4.7.

Both TMA and VB increased with storage. TMA increased from an initial value of 3.4 mg/lOOg to 4.9-5.3 mg/100g after 3 days of storage and continued to increase up to 21 days reaching 7.3-7.9 mg/100g in all silages made from different carbohydrates with different type of inocula. Rapid increase in VB production was noticed in all the samples. VB increased from 39.4 mg/lOOg to 155 - 162.5 mg/100g after 3 days of storage and reached 387.8 to 406.1 mg/100g in 21 days.

Total bacterial count on MRS agar increased in all silages during 3 days fermentation. The range of bacterial count in all silages was 9.1 x 10^8 to 9.2 x 10^8 CFU/g on the third day of storage whether inoculated with *L.plantarum* or sauerkraut and continued to decrease to a value of 1.3 x lO^5 - 1.4 x 10^8 CFU/g after 21 days.

The microbial silage prepared using sauerkraut as starter with different carbohydrate sources produced slight off odours during the early period of fermentation (1-2 days) compared to silages prepared by using lactic acid bacteria as starter. After 3 days an agreeable odour was noticeable in all silages becoming pleasantly cheesy, or buttery, after 21 days of ensilation.

Figures 3 and 4 present the chemical changes in insoluble N, NPN and NH_3 -N in all silage samples. There also appears to be no noticeable difference in the parameters studied. All the samples liquefied in 3-7 days. The NPN (as percent of total nitrogen) increased throughout 21 days of storage. At the start of the experiment, there was around 90% TCA insoluble N, about 10% TCA soluble N or NPN and traces of NH_3-N in the fermentation mixture. The NPN increased to around 36% (35.9 to 36.6%) after 3 days of fermentation and reached a value of 60.3 to 63.4% after 21 days.

NH_3-N showed the same trend, rising from traces to a value of 6.25 - 6.5% after 3 days and 15.8 to 16.5% after 21 days of storage in different silage samples.

Comparison of Acid and Microbial Silage

Initially there was no significant difference in the value of TMA, VB, insoluble N, NPN and odour in acid and microbial silages. However, the pH of microbial silage was 6.0 and that of acid silage 4.0 due to addition of formic acid. The bacterial count of acid silage was 10^6 CFU/g, the corresponding count of microbial silage was more than 10^7 CFU/g, due to inoculation of lactic acid bacteria. Figure 5 shows the changes in pH, TMA, VB and bacterial counts of fermented and acid ensilage at 3, 7, 14, 21, 30, 60, 90, 120 and 365 days. In acid silage the pH remained almost constant throughout the year. On day 3,the pH was 4.0. There was slight decrease afterwards and the pH dropped to 3.85 after 14 days. After 30 days of storage the pH again rose to 4.0.

In case of microbial silage the pH decreased rapidly from an initial value of 6.0 to 4.25 within 3 days and thereafter remained constant up to 7 days. After 7 days, there was slight increase and the PH rose to 5.0 after 30 days. There was a very slight increase, if at all, after this period and the pH never reached more than a value of 5.1.

The production of TMA and VB in fermented silage was higher as compared to acid silage throughout the year. The TMA increased from an initial value of 0.95 mg/100g to 7.8 mg/lOOg in acid silage and 22.5 mg/1OOg in fermented silage in one year. A prominent difference was noticed in the production of VB. In fermented silage VB increased from 13.2 gm/100g to 98.5 mg/lOOg after 3 days of storage and continued to increase to a value of 925.5 mg/100g in one year. In acid silage, a slow increase in VB was noticed, and it reached to a level of 50.2 mg/100g in one year. Higher values of VB in fermented silage were attributed to the production of excessive amounts of NH_3 as is evident from Fig. 6.

In acid silage, the bacterial counts decreased from an initial value of 1,1 x 10^5 to 9.1 x 10^4 CFU/g within 3 days, further decreased to 2.9 x 10^3 CFU/g after 30 days and continued to decrease slowly throughout the storage reaching a value of 1.0 x 10^3 CFU/g after one year. In microbial silage, the bacterial count increased from 10^7 to 9.7 x 10^9 CFU/g within 3 days of ensilation. There was a significant decrease afterwards and the count dropped to 7.0 x 10^6 CFU/g after 30 days. The bacterial count continued to decrease further during storage dropping to a value of 1.3 x 10^3 CFU/g after one year. These results are in agreement with the findings of James *et al* (1977).

Figures 6 and 7 present the degradation of protein nitrogen during the storage of acid and microbial silages at 3, 7, 14, 21, 30, 60, 90, 120 and 365 days of storage. Although the liquefaction was complete within 3-7 days, the protein hydrolysis continued to occur for at least one month in both the silages. No degradation of protein was observed during further storage.

At the start of ensilation, there was around 92.5% insoluble N around 7.5% NPN and traces of NH_3 in both acid and microbial silage mixtures. In acid silage NPN increased to 46% from the initial value after 3 days, to 84% after 30 days, whereas in fermented silage the corresponding value of NPN was about 31% after 3 days and about 69% after 30 days. After one year storage NPN increased to about 85% in acid silage and 74% in microbial silage.

The production of NH_3 in fermented silage was considerably higher than acid silage. In acid silage, NH3 produced was 0.5% after 3 days and reached to a value of 0.8% after 30 days. After one year it was 1.8%. In contrast, a rapid production of NH_3 was noticeable in microbial silage The corresponding values were 4% after 3 days, 18.1% after 30 days and 37.5% after one year. A rapid production of NH_3 in fermented silage has also been reported by Kompiang *et al* (1979). They concluded that the difference between chemical and microbial silage in NH_3 production might be, at least, partly due to different methods of storage.. In this study chemical and microbial silages were stored under similar conditions and yet same results were obtained. Brady (1966) and McDonald & Wittenbury (1973) reported that amino acids are one possible source of energy for lactic acid bacteria. Therefore, high NH_3 content in microbial silage might be due to deamination of amino acids by lactic acid bacteria.

The fish silage prepared by the addition of formic acid gave pungent odours, while microbial silage gave highly pleasant odours throughout the storage.

Many studies have shown that 10% carbohydrate level with minced fish gave a stable silage. Kompiang *et al* (1979) used different ratios of molasses with fish and reported that fish molasses ratio of 100:5 was inadequate for silage preparation, where the pH never dropped below 5.0. The silage with fish molasses ratio of 100:10 also spoiled within 2 to 3 weeks but the silage prepared with fish: molasses ratio of 100:15 and 100:20 remained stable with a pH value of 4.3 and could be stored longer. James *et al* (1977) used 15% molasses by weight of fish for the production of microbial silage, whereas, Roa (1965) successfully prepared silage by using 10% molasses.

In the light of these reports, in preliminary trials, 10, 15 and 20% molasses was used from different sources. It has been observed that 10% molasses is the best for preparation of fermented fish silage as far as odour and economy are concerned. In silage with 15 and 20% molasses the smell of molasses was very dominant and pleasant odour of microbial silage was suppressed. Therefore, only 10% molasses was used in the study. Molasses obtained from different sources gave different results. It was observed that whenever silage was prepared from molasses of a certain source putrefaction started within 2 weeks. In contrast, using the molasses from another source, stable silage was prepared several times. This indicates that silage preparation depends upon the quality of molasses used perhaps on the sucrose content. It is, therefore, recommended that to prepare stable silage 5% molasses along with 5% other carbohydrate source either sucrose or glucose should be used. This will take care of fluctuations in carbohydrates content of molasses samples. This observation prompted us to prepare silage using 5% molasses and 5% sucrose for further studies (unpublished) and for the comparison of acid and microbial silage.

These studies show that stable microbial silage can be prepared by using either 10% (w/w) quantity of sucrose or liquid glucose or good quality molasses. Similar results may also be obtained by using 5% molasses and 5% of either sucrose or liquid glucose. The results are in agreement with the findings of Hercules *et al* (1985), who reported that silages made from mixtures containing a total carbohydrate content of 10% were the best to prepare stable silage.

The results of the comparative study of two inocula clearly indicate that sauerkraut (4-8 days fermented cabbage with a pH 4.0 or below) can be used safely as starter inoculum for microbial silage preparation. Disney *et al* (1977) pointed out that maintenance of starter culture at a village level in tropical countries is a serious handicap in the preparation of fermented silage. Stanton & Yeoh (1977) drew their bacterial inoculum from 4 days old sauerkraut juice, for fish silage preparation. In these studies 4-8 days fermented cabbage with a pH 4.0 was used for inoculation, since the fermented cabbage is an edible product and can safely be used in silage preparation.

Hercules *et al* (1985) reported that lactobacilli differ in their ability to stabilize fish silage. Though in this study different *Lactobacillus* cultures were not compared for their fermenting capabilities, a comparative study using strain of *Lactobacillus plantarum* and 4-8 days stored sauerkraut as inoculum was made in detail during 21 days of storage. Not much difference was observed in fermentation behaviour of pure culture of *L.plantarum* and sauerkraut as inoculum. Sauerkraut usually contain natural flora of lactobacilli comprising of *L.brevis, L.casei, L.plantarum* and *Leuconostoc* spp. However, the dominating organisms are usually *L.plantarum* or *L.casei*, as reported by Pederson (1960). This may explain the similarity in the behaviour of sauerkraut and *L.plantarum* as inoculum. The merit of sauerkraut as inoculum was studied in detail and compared with pure culture of *Lactobacillus*. No noticeable difference was observed in the quality of silages during 21 days of storage.

For the preparation of sauerkraut, availability of cabbage can not be assured throughout the year, the price fluctuation may also affect the economics of the process. This problem may also be solved by selecting easily available pulses such as green gram (*Phaseolus aureus*) and black gram (*Phaseolus mung*). The starter may be prepared using the whole seed or paste of soaked pulses containing 2.5 NaC1. Fermented fish silage can also be prepared using 10% (w/w) fermented pulse as starter.

To prevent mould and yeast growth the walls of the containers and surfaces may be sprayed with propionic acid or 1% potassium sorbate after 24 hrs of fermentation. Usually 10^8 CFU/g of lactic acid bacteria are produced within 4-8 days at 28-30°C incubation by natural fermentation and pH drops to 4.0.

Our studies have shown that either sauerkraut or fermented green gram can be used as starter. The choice will obviously depend on the availability and cost of material used. In fact, the process of preparation of sauerkraut and fermented green gram pulse is very simple.The only precaution to be taken is that sauerkraut or fermented green gram should be maintained at pH 4.0 or below, and ensuring the absence of yeast and mould. A semi skilled worker can do the job.

CONCLUSIONS

It may be concluded that a stable fish silage can be prepared either by addition of formic acid or by fermentation. The microbially fermented fish silage can be prepared, within 3-4 days at 28-30°C, by adding fermentable carbohydrate source up to 10% along with 10% broth culture (v/w) of *L.plantarum* or 10% (w/w) 4-8 days old sauerkraut or 4-8 days old fermented green gram pulse as starter. On the basis of cheesy and buttery odour, microbial silage is more acceptable than acid silage. Microbial silage does not require the acids which are potentially dangerous and expensive.

Furthermore, the availability of acid at village level may itself be a problem. For microbial silage, the maintenance of starter culture at village level may not be a problem. Sauerkraut and fermented green gram can be used as a substitute for starter culture, which can easily be handled at a village level. Long storage of microbial silage in liquid form is not advisable due to high ammonia production. It should preferably be dried within one week. A mixture of 5% sucrose and 5% molasses is probably the safest and cheapest carbohydrate source for the preparation of fermented silage.

Preparation of microbial fish silage, though does not require total aseptic conditions for fermentation, certain precautions are necessary for good fermentation. Minced fish carbohydrate and starter need to be thoroughly mixed. Yeast and mould growth should be avoided at all cost. This can be achieved by spraying propionic acid or 1% solution of potassium sorbate on surface of silage and on the walls of the container.

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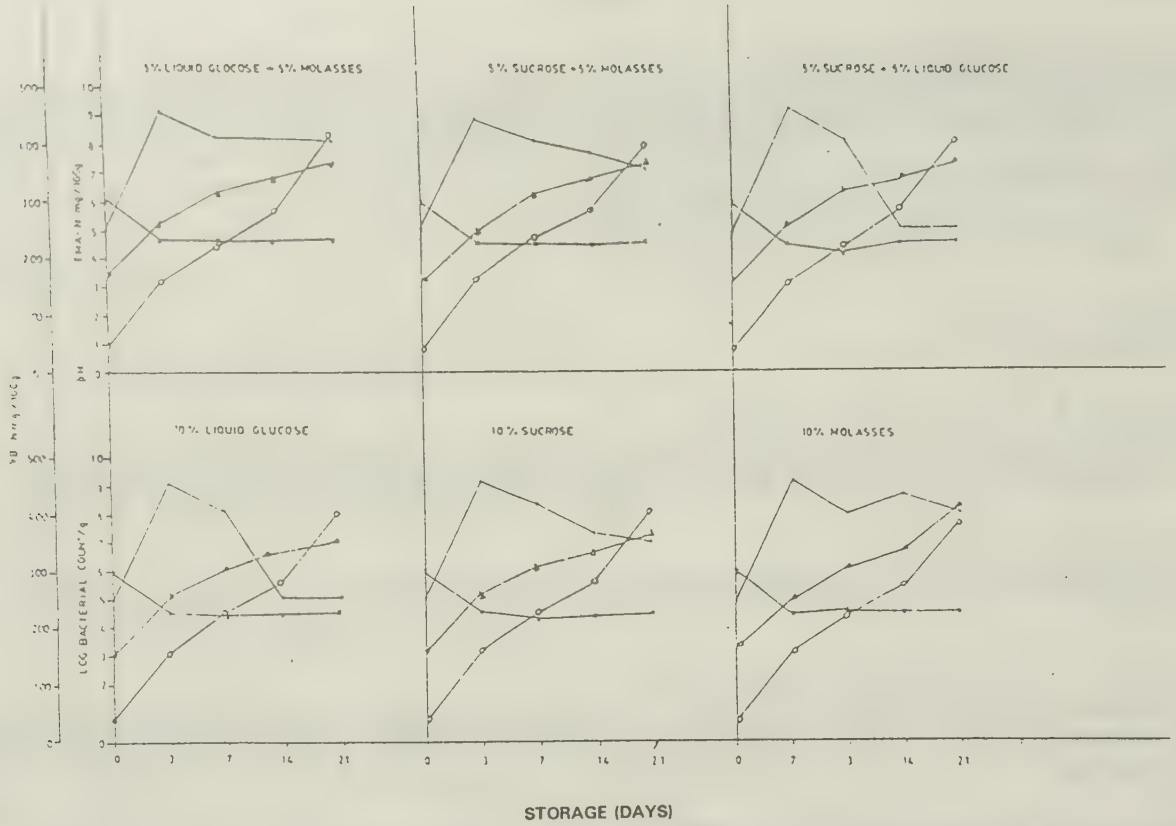

FIGURE 1 CHANGES IN MICROBIAL FISH SILAGE DURING PREPARATION WITH DIFERENT CARBOHYDRATE SOURCES INOCULATED WITH *LACTOBACILUS PLANTARUM* AS STARTER: ▲——▲ BACTERIAL COUNT. ■——■ pH △——△ TMA.N □——□ VB.N.

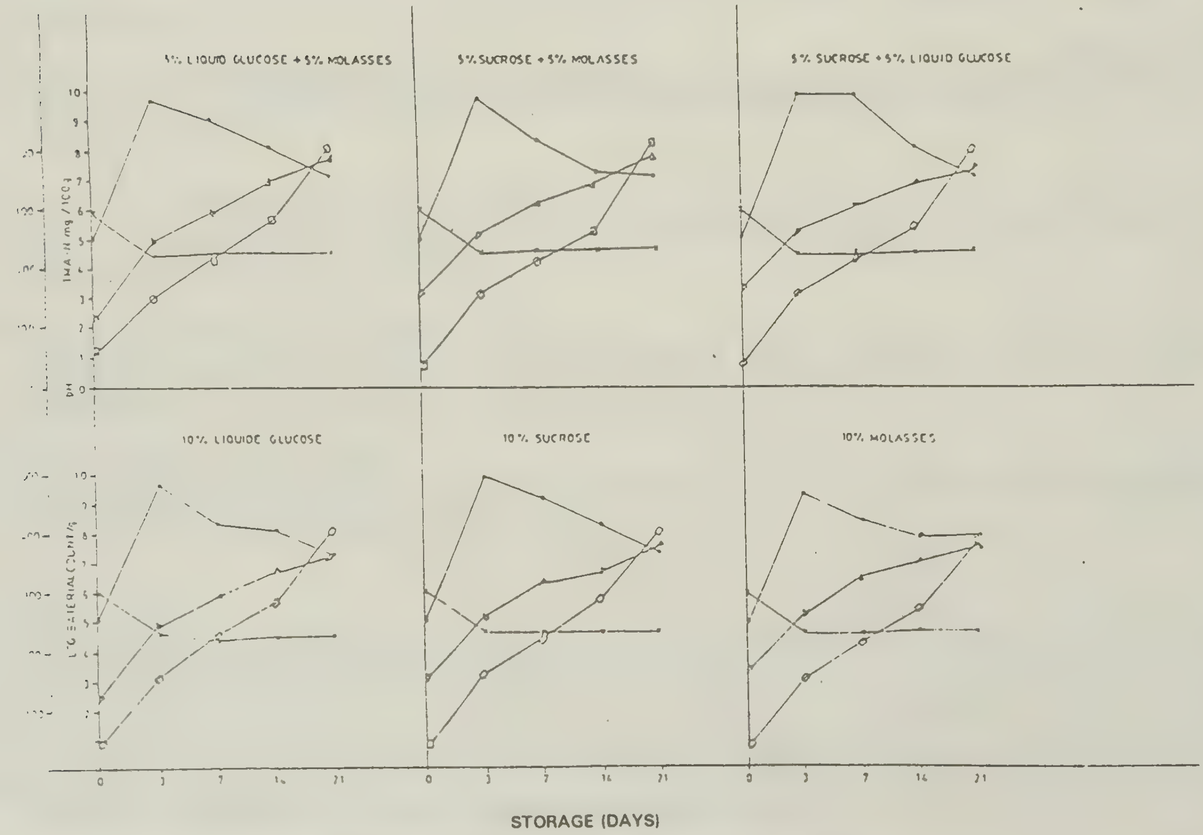

FIGURE 2 CHANGES IN MICROBIAL FISH SILAGE DURING PREPARATION WITH DIFFERENT CARBOHYDATE SOURCES INOCULATED WITH SAUERKRAUT AS STARTER: ——▲ BACTERIAL COUNT. ■——■ pH △——△ TMA.N □——□ VB.N.

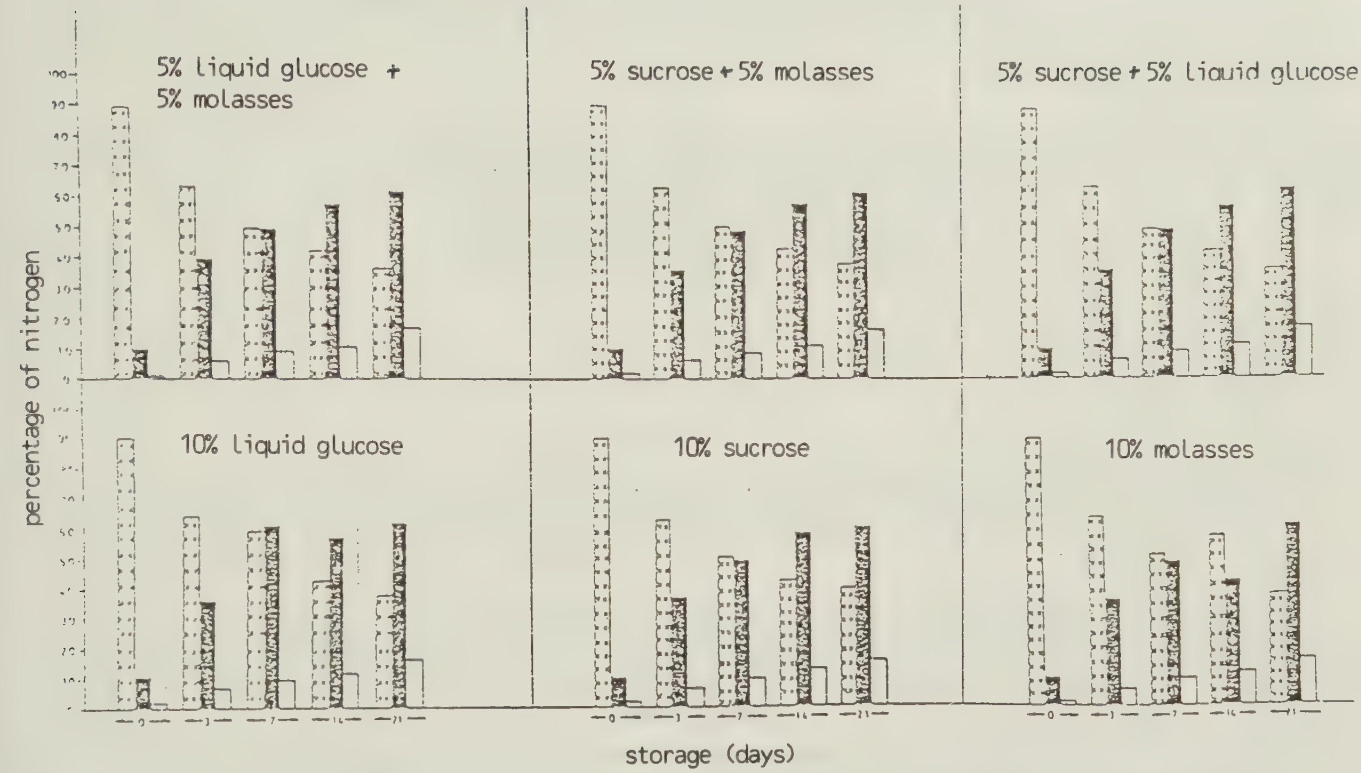

FIG. 3 PROTEIN DEGRADATION IN MICROBIAL FISH SILAGE DURING PREPARATION WITH DIFFERENT CARBOHYDRATE SOURCES INOCULATED WITH *LACTOBACILLUS PLANTARUN* AS STARTER. ▢ INSOLUBLE NITROGEN, ▦ NON PROTEIN NITROGEN, ▢ AMMONIA

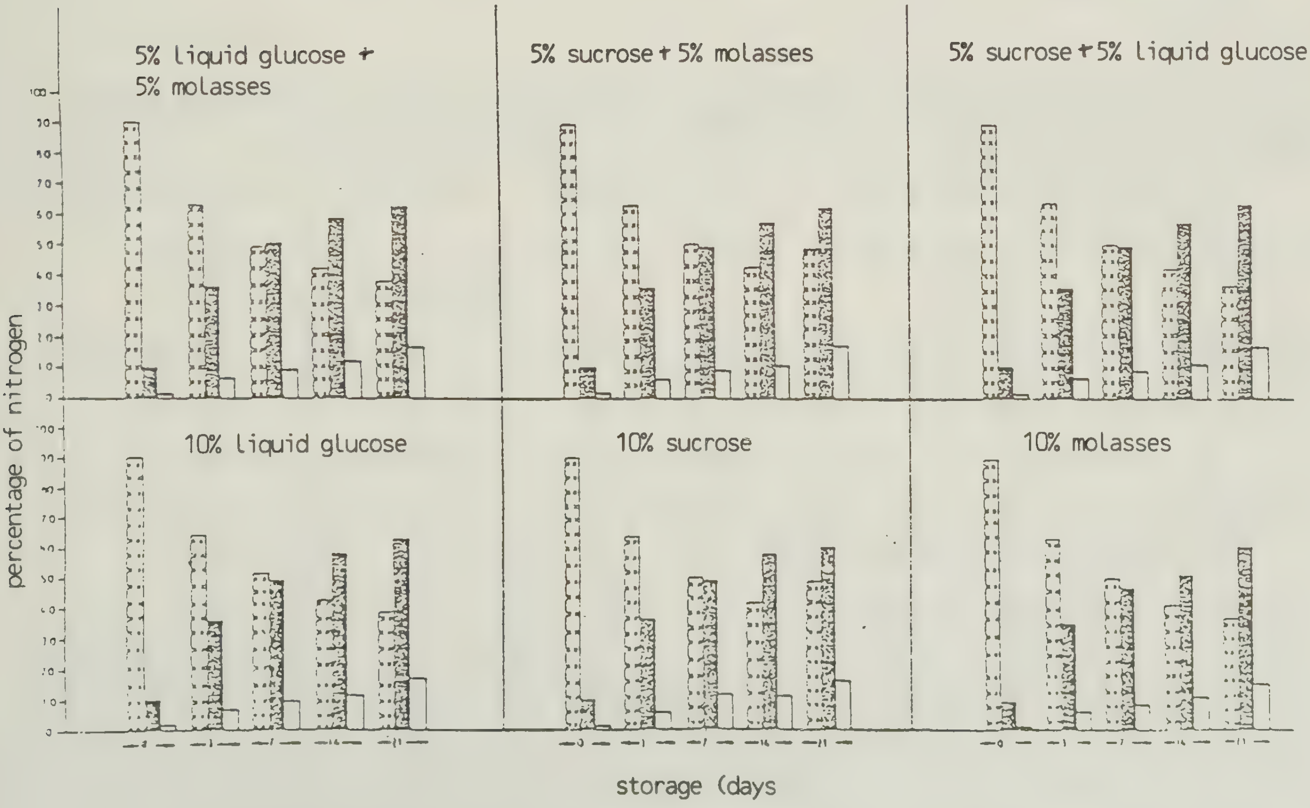

FIG. 4 PROTEIN DEGRADATION IN MICROBIAL FISH SILAGE DURING PREPARATION WITH DIFFERENT CARBOHYDRATE SOURCES INOCULATED WITH SAUERKRAUT AS STARTER. ▢ INSOLUBLE INTROGEN, ▦ NON PROTEIN NITROGEN, ▢ AMMONIA

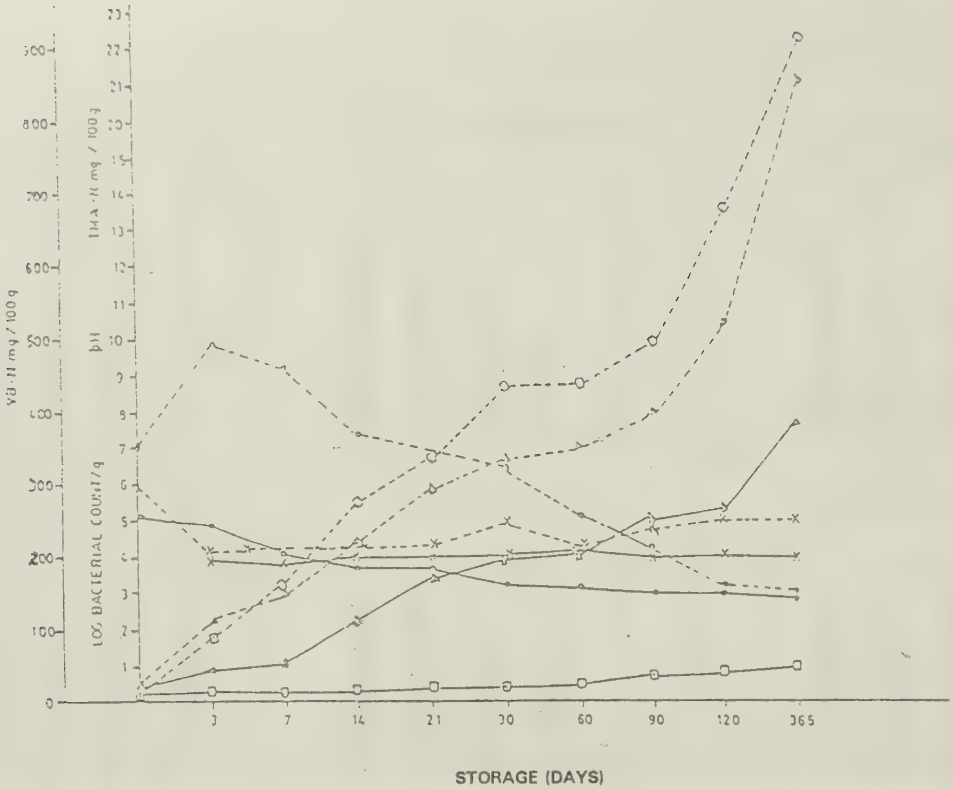

FIGURE 5 CHANGES IN MICROBIAL AND ACID FISH SILAGE OVER A PERIOD OF ONE YEAR MICROBIAL FISH SILAGE: ○-----○ BACTERIAL COUNT, x-----x pH, △-----△ TMA.N, □-----□ VB.N.
ACID SILAGE: ○——○ BACTERIAL COUNT, x——x pH, △——△ TMA.N, □——□ VB.N.

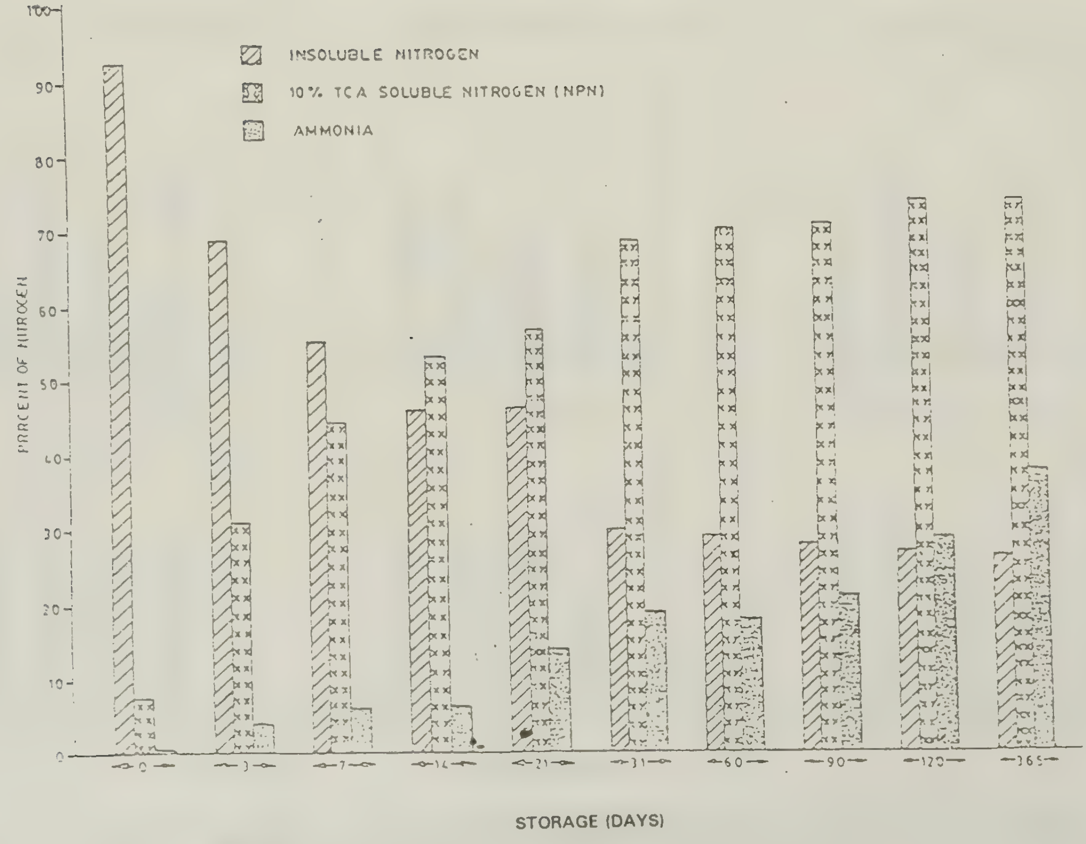

FIGURE 6 PROTEIN DEGRADATION IN MICROBIAL FISH SILAGE DURING STORAGE

FEEDING STUDIES ON TILAPIA (*Oreochromis sp.*) USING FISH SILAGE

by

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ABSTRACT

Fish silages were prepared from ground fish offals (heads, tails, fins, viscera and trimmings of Sardinella spp.) by addition of concentrated formic acid (98%) at an amount equivalent to 2.5, 3.0 and 3.5 v/w of the fish offal.

The final product chosen for feed formulation was the sample with the lowest concentration of formic acid (2.5%) since the Analysis of Variance (ANOVA) indicated no significance difference in amino nitrogen content among the samples at 5% level.

The efficiency of the diets formulated from fish meal and those from a combination of fish silage and fish meal were compared by determining the growth response in tilapia. The diets were isoproteinic. The results showed that Diet I (with fish meal) and Diet 2 (1:1 fish meal fish silage) were more efficient than Diet 3 (1:3 fish meal fish silage) as exhibited by higher average weight gain, percent weight gain and specific growth rate value. The highest Protein Efficiency Ratio (PER) was obtained in tilapia fed with Diet 2.

INTRODUCTION

There is a growing interest to introduce the silage process in both industrialised and developing countries mainly because fish silage can be a means of utilising waste fish in situations where conventional fish meal production is inappropriate. However, fish silage may also become a competetive process under conditions which traditionally have been favourable for fish meal due to a number of advantages:

a) Acid preserved fish meal does not putrefy, retaining a fresh acidic smell even after storage for weeks at tropical temperature;

b) The scale of production of fish silage can be varied at will without the economy of the process being much affected;

c) Fish silage is almost sterile and pathogens like salmonella are efficiently killed in it;

d) Mixtures of acid preserved fish silage and carbohydrate fillers can be sun dried in open trays under tropical conditions without fly infestation because flies are repelled by the evaporating acids.

Since interest in fish culture as a means of producing human food has increased, the trends in fish farming have been geared towards intensification of production where large numbers of aquatic animals are reared in relatively restricted areas. In these culture systems, the contribution of natural food organisms to the dietary requirements of the animals become insignificant. The supplemental feed that must be supplied is the single most critical and expensive commodity in the entire operation. In the intensive culture systems, the cost of feed alone may represent 50% or more of the total production cost. Consequently, development of acceptable, nutritionally adequate and least cost diets suitable for various stages of the species cultured and different systems is essential to the success of fish farming.

Dietary protein is always considered to be of primary importance in fish feed as the protein requirement of fish are higher from those of terrestial animals and protein sources account for more than half of the total feed cost. Protein is the basic component of the fish body.

Diets based on fish silage have been succesfully used for trout in Norway (Asgard, 1981, Raa and Gilberg 1980) although no data appear to exist for tilapia. Observations at the University of Stirling indicate that tilapia prefer smaller pellets than the carp and salmonids.

The study was done to determine the optimum proportion of fish silage to fish meal to be incorporated in tilapia diets, and to compare the efficiency of diets formulated from fish meal and those from fish silage and fish meal as feed for tilapia. Parameters such as percentage weight gain, specific growth rate, feed conversion efficiency, protein efficiency ratio and apparent net protein utilisation were used.

MATERIALS AND METHODS

Feeding Studies

Feed Formulation

Tilapia diets were formulated from dried fish silage and fish meal. Three types of diet were used namely: Diet 1 (control) , Diet 2 (1:1 ; fish meal :fish silage) and Diet 3 (1:3; fish meal:fish silage). The ratio refers to the proportion of dietary protein from either meal or silage. The formulation was based on the results of the Apparent Digestibility Coefficient of fish meal and fish silage. Therefore, the computed energy values represent the digestible energy. The diets were formulated in such a way that the three types of diets were isonitrogenous, that is, all contained 25% digestible protein (Appendix G). Since the digestible protein level of the diet is 25%, 12.5% of this was derived from fish meal and 12.5% from fish silage for 1:1 ratio. For 1:3 ratio, 6.25% was from meal and 18.75% from silage. In the control diet, the dietary protein came mainly from fish meal and partially from rice bran. The total digestible energy values of the diets ranged from 250.84 to 252.08 kcal/100g.

Other ingredients included in the diet aside from fish meal, fish silage and rice bran were vitamin/mineral premix (Afsilin), oil, (combination of vegetable and animal fat, 1:1 ratio), and corn starch.

After all the diet components have been weighed, sieved (solid ingredients), mixed and pelletized, they were cabinet-dried at a temperature of 58°C-60°C to moisture content of 10%-15%. The moisture of the diets was closely to determine whether the desired moisture content was attained.

Proximate composition of the experimental diets are shown in Table 1.

Feeding Trials

Nine rectangular all-glass aquaria (cap. 60 gallons) each provided with continous aeration were used in these feeding experiment. About 15 *Oreochromis* fingerlings were stocked per aquarium. The fingerlings had an average weight of 1.30g for Trial I, 0.60g for Trial II and 4.03g for Trial III.

Prior to the feeding experiment, the fish were fed twice daily, in the morning (9:00 am) and in the afternoon (4:00 pm) for one week. All the fish were weighed at the beginning of each experiment and at weekly intervals thereafter for eight weeks. Weighing was done by getting the weight of plastic container with water followed by placing the fish inside the container and weighing again. The difference between the weight of the container with water and fish and the weight of container with water only represents the weight of the fish. The experimental diets were given to the fish little by little to minimize errors in measuring feed intake due to unconsumed feed. The diets were crumbled first before feeding since the tilapia used were of smaller size. The aquaria were cleaned once every two days and whenever the fish were removed for weighing.

The pH of the water in the aquaria was measured to determine the effect of acid-treated silage on the water.

The following parameters were determined to assess feed efficiency:

1. Growth rate:

a) percentage weight gain

$$= \frac{\text{mean final fish wt.} - \text{mean initial fish wt.}}{\text{mean initial fish wt.}} \times 100$$

b) specific growth rate, SGR (%/day)

$$= \frac{\log_e W_2 - \log_e W_1 \times 100}{T_2 - T_1}$$

where W_2 is the weight at time T_2 and W_1 the weight at time T_1 with the weight and time usually expressed in grams and days respectively. This gives the average percentage increase in body weight per day over any given time interval.

2. Feed Conversion Efficiency (FCE) gives the weight gain of fish per unit wt. of feed. In determining FCE, the initial and final fish weight and the weight of feed taken in by the fish for a specific period was recorded.

$$\text{FCE} = \frac{\text{weight gain}}{\text{feed intake}}$$

3. Protein utilization

a) Protein Efficiency Ratio, PER

$$\text{PER} = \frac{\text{weight gain}}{\text{protein intake}}$$

PER gives the weight of fish produced per unit weight of dietary protein. It is measured from the gain in weight (final wt. - initial wt.) of the fish and feed intake for a specified period of time. Protein intake is computed based on the weight of feed intake multiplied by the % protein level of the feed.

b) Apparent NPU (%)

$$\text{NPU} = \frac{B - B^o}{I} \times 100$$

where B is the total fish body protein at the end of a growth period of fish fed a protein intake I, and B^o is the initial body protein.

Apparent NPU value estimates the percentage of ingested protein retained.

The value for growth rate, feed conversion efficiency and protein utilization were treated statistically using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Feeding Trials

Feeding experiments have been carried out with tilapia to compare the value of different ingredients or combinations of ingredients. The average size of tilapia fingerlings used in this experiment ranged from 1.30g for first trial, 0.69g for the second trial to 4.03g for the third trial. For tilapia ranging from 0.5-35g, a crude protein level of about 35% is required for optimum growth (Jauncey and Ross, 1982). This protein level is very difficult to attain in fish silage since the crude protein content of silage after sun drying was only

31.52%. Much more, after the addition of other ingredients in the formulation, the crude protein level decreased further to 27.80% for Diet 2 and 26.78% for Diet 3 (Average of 3 trials). These conditions prevented the preparation of a diet using fish silage as the sole source of protein.

Jackson & Copper (1982) reported that the quantitative dietary protein and amino acid requirements of tilapia appear to be lower than those of other cultured species. Hence, the sub-optimal protein level of the experimental diets may not be such a disadvantage. Moreover, in a study made by Jauncey and Ross (1982) on *O. mossambicus*, it was found that for fish weighing 0.5-10g, a protein level of 24% still produced 80% of the maximum growth rate and even a protein level of 16% resulted in 64% of the maximum growth rate.

Feed Intake

During the first, second and third trials of the feeding experiment, fish given the control diet (Diet 1) accepted their rations and fed more aggressively than fish fed with either of the other two diets as shown by the higher average feed intake of the former than the latter two (Table 2). While the average feed intake of Tilapia fed with Diet I was about 8g for trials I & II, it was only about 6g for both Diet 2 and 3. The same trend was observed in the third trial. Regardless of trial the difference in feed intake between Diets I and 2 was significant at 5% level. A higher feed intake was noticed in trial III which was ascribed to the larger size of fish used.

The low intake of Diets 2 and 3 could be attributed partly to the texture of the pellets. These feeds appeared to be slightly moist or oily because of higher fat content compared with Diet I (Table 1). The higher fat content in Diets 2 and 3 could have also adversely affected the palatability of the diets. In a preliminary study by Arifudin *et al.* (1978), it was observed that aqueous extracts of silage has a pungent and bitter taste believed to be due to peptides which form during proteolysis, and to lipids.

Growth

The weekly average gain in weight of fish fed with experimental diets are shown in Figures 1 to 3. In trials I and III, tilapia fed with the control diet exhibited the highest weight gain followed closely by those fed with the second and third diets respectively. The apparent weight gain between Diets I and 2 was not significant at 5% level. In the second trial, tilapia fed with Diet 2 registered the highest weight gain followed closely by those fed with the control diet. Again, the difference in weight gain between these two diets was not significant. Both Diets 1 and 2 resulted in weight gains significantly different from that in Diet 3, regardless of trial.

The percent gain in weight was highest in Diet 2 for all the trials (Table 3). Therefore, the average SGR is also highest in Diet 2. When the SGR values were treated statistically, the difference between Diets 1 and 2 was not significant at 5% level. However, both Diets 1 and 2 resulted in SCR values significantly different from that in Diet 3.

Results of all trials indicated that the growth response of tilapia to Diets 1 and 2 were almost the same and better than Diet 3. Both the percent weight gain and average SGR values were higher in trial II than in trials I and III. The difference in the size of fish used may have played a major role i.e. as the fish grow older, the rate of growth decreases (Brown, 1957).

Appler and Jauncey (1983) found better growth response on tilapia (*Sarotherodon niloticus*) fingerlings fed with diets containing digestible protein from 27-29% than those obtained in this study. The higher growth response could be due to the higher digestible protein level of the diets since in the present study, the formulated diets contained only 25% digestible protein. Moreover, sampling was done once every two weeks in the study of Appler & Jauncey (1983) while in the present, it was done once every week, and the fish was subjected to more stress. This increase in fish activity as a result of physical handling increases energy requirement and can adversely affect growth rate (Phillips, 1972).

Feed Conversion

In the first two trials, Diet 2 was more efficiently utilized by the fish than Diet 1 and 3 which showed almost equivalent feed efficiency, expressed as FCE in trial II (Trial 2). The results indicated that a smaller amount of Diet 2 is needed to produce an equal gain in weight as those in Diets 1 and 3. In trial III, FCE of Diet 3 was higher than that of Diet 1. The difference in FCE between Diet 1 and 2 maybe partly due to higher fiber content of Diet 1 (Table 1). According to Welch (1968), presence of a nondigestable fraction, in significant amounts could lower feed efficiency. Rice bran was mainly responsible for the higher fibre content of Diet 1 since the fish meal used contains 1.5% fiber only. For trial I, the FCE values of the three diets were significantly different from each other while in trial II, the FCE of Diet 1 and 3 were significantly different from Diet 2. On the other hand, no significant difference was observed in Trial III. The FCE values in this study were lower compared with the results of Appler and Jauncey (1982).

Protein Utilization

The protein efficiency ratio (PER) of the three diets were determined (Table 4). The highest PER value was obtained for Diet 2 in all the three trials. This would mean that protein in Diet 2 was more efficiently utilized by tilapia as shown by greater weight gain per unit weight of dietary protein. For the first two trials, PER values of Diets 1 and 3 are quite close and the difference between them was not significant at 5% level. On the other hand, the difference in PER between Diets 1 and 2 and between Diets 2 and 3 were significant. In trial III, the difference in PER among the three diets was not significant. Higher PER values were obtained in trial II as a result of better growth response than in the other two trials.

In the measurement/calculation of PER, it is possible that the weight gain is due to increased lipid and/or moisture contents instead of increased protein deposition in the carcass. These variations in carcass composition are taken into account of the apparent Net Protein Utilization (NPU). Hence, the initial and final carcass composition of tilapia were determined (Table 5). Balarin and Hatton (1979) summarized published data on the carcass crude protein content of tilapia as between 7 and 16%. The protein contents reported in the experiment were found to be at the upper end of this range. Due to some inconsistencies in the withdrawal of samples for protein analysis, i.e. the whole fish was used for initial protein analysis while for the final analysis, the fish less the head and tail was utilized, the apparent NPU of Diets 1-3 in the first two trials cannot be determined. For the third trial, the highest NPU value of Diet 3 was only slightly lower than Diet 2 while the lowest value was observed in Diet 1. A number of authors have reported increasing PER and NPU with decreasing dietary protein level (Ogino and Saito, 1970; Dabrowski, 1977; Mazid *et al.*, 1979; Jauncey, 1981; 1982) for various species. A similar pattern was observed in the results obtained in this paricular study. As a result of higher protein digestibilities of fish silage than fish meal, the diets with fish silage have a lower dietary protein level.

ACKNOWLEDGEMENT

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Table 1. Proximate composition of experimental diets

Diet*	Component, g/l00g diet					
	Protein	Moisture	Fat	Ash	Fibre	NFE**
Trial I						
1	29.56	12.93	10.05	13.68	9.41	24.37
2	27.89	13.86	12.39	15.93	2.25	27.68
3	25.80	13.51	12.84	15.24	1.78	19.83
Trial II						
1	29.24	13.47	9.80	14.83	9.50	23.16
2	27.22	14.59	12.04	16.19	2.10	27.86
3	26.55	14.81	12.67	15.17	2.03	28.77
Trial III						
1	20.45	11.67	10.55	13.12	8.76	25.45
2	28.30	13.01	12.43	15.22	1.93	29.11
3	27.00	13.32	13.67	13.65	1.74	30.62

* 1 - control
2 - 1:1, fish meal: fish silage
3 - 1:3, fish meal: fish silage

** Nitrogen-free extract

All values are average of 3 trials.

Table 2. Feed intake and feed conversion efficiency (FCE) of tilapia on different diets.

Diet	Average Feed Intake g	FCE
Trial I		
1	8.02^a	0.27^a
2	5.60^b	0.38^b
3	5.75^b	0.20^c
SEM	0.30	0.01
Trial II		
1	8.64^a	0.31^a
2	5.95^b	0.51^b
3	5.60^b	0.30^a
SEM	0.12	0.04
Trial III		
1	22.14^a	0.29^a
2	16.16^b	0.35^a
3	13.49^b	0.33
SEM	0.73	

* 1 - control
2 - 1:1, fish meal: fish silage
3 - 1:3, fish meal: fish silage

Figures with the same superscripts in a specific trial are not significantly different (P 0.05).
All values are average of 3 trials.
SEM - Standard mean deviation

Table 3. Growth rate of tilapia on different diets

Diet*	Mean initial weight (g)	Mean final weight (g)	Weight gain (g)	Percent weight	Average SGR, % per day
Trial I					
1	1.51^{a}	3.671^{a}	2.16^{a}	143.35	1.59^{a}
2	1.38^{a}	3.51^{a}	2.12^{a}	153.48	1.66^{a}
3	1.36^{a}	2.48^{a}	1.12^{b}	82.89	1.08^{b}
SEM		0.16			0.05
Trial II					
1	0.73^{a}	3.38^{a}	2.66^{a}	364.27	2.74^{a}
2	0.75^{a}	3.77^{a}	3.02^{a}	404.75	2.89^{a}
3	0.71^{a}	2.38^{b}	1.67^{b}	236.59	2.17^{b}
SEM		0.23	0.25		0.09
Trial III					
1	4.13^{a}	10.55^{a}	6.42^{a}	155.47	1.67^{a}
2	3.66^{a}	9.34^{a}	5.68^{a}	155.85	1.68^{a}
3	4.11^{a}	8.50^{b}	4.39^{b}	109.35	1.31^{b}
SEM		0.31	0.24		0.06

* 1 - control
2 - 1:1, fish meal: fish silage
3 - 1:3, fish meal: fish silage

Figures with the same scripts in a specific trial are not significantly different (P=0.05)

All values are average of 3 trials.

SEM - Standard mean deviation

Table 4. Protein efficiency ratio (PER) and apparent net protein utilization (NPU) of tilapia fed with experimental diets.

Diet*	PER	Apparent NPU, %
Trial I		
1	0.91a	-
2	1.36b	-
3	0.73a	-
SEM	0.05	-
Trial II		
1	1.05 a	
2	1.87b	
3	1.13b	
SEM	0.14	
Trial III		
1	0.95a	0.65a
2	1.25a	0.85b
3	1.21a	0.03

*1 - control
2 - 1:1, fish meal: fish silage
3 - 1:3, fish meal: fish silage

Figures with the same super scripts in a specific trial are not significantly different (P=0.05)

All values are average of 3 trials.

SEM - Standard mean deviation

Table 5. Percent body composition of tilapia fed with experimental diets

Diet*	Protein		Moisture		Fat		Ash	
Trial I	Initial/Final		Initial/Final		Initial/Final		Initial/Final	
1	13.02	16.29	78.61	76.82	2.53	2.34	5.37	4.33
2	12.92	15.24	79.03	77.43	2.37	3.34	5.20	3.84
3	13.25	15.86	77.34	77.06	3.48	3.84	5.59	3.13
Trial II								
1	13.03	16.42	77.80	77.20	2.57	4.14	4.45	2.20
2	14.24	16.83	77.42	76.95	2.84	3.94	5.12	2.03
3	14.32	16.89	77.46	76.43	3.01	4.08	4.75	2.40
Trial III								
1	12.58	16.94	70.32	76.62	2.51	2.55	5.29	3.64
2	12.67	16.53	78.56	75.93	2.71	3.48	5.86	3.73
3	12.74	15.77	78.40	76.54	2.83	3.76	5.81	3.65

*1 - control
2 - 1:1, fish meal: fish silage
3 - 1:3, fish meal: fish silage

All values are average of 3 trials

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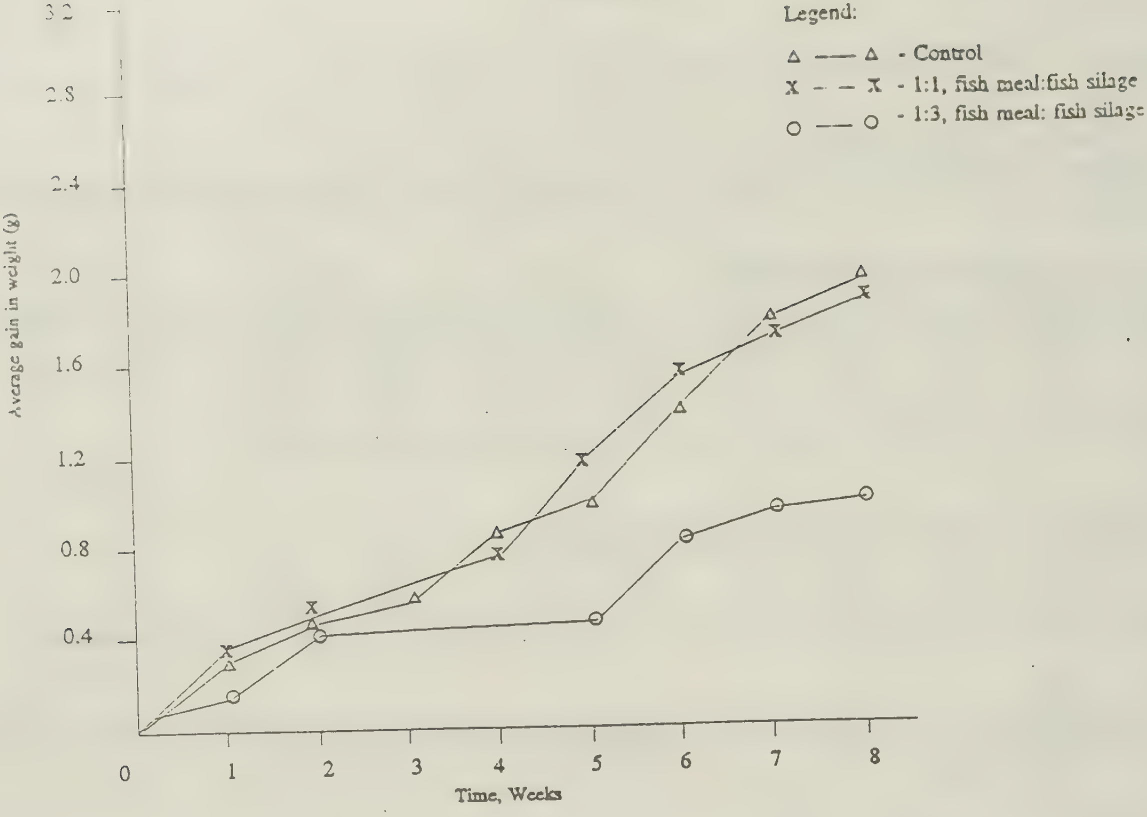

Figure 1. Average gain in weight of fish in 8 weeks feeding (Trial I)

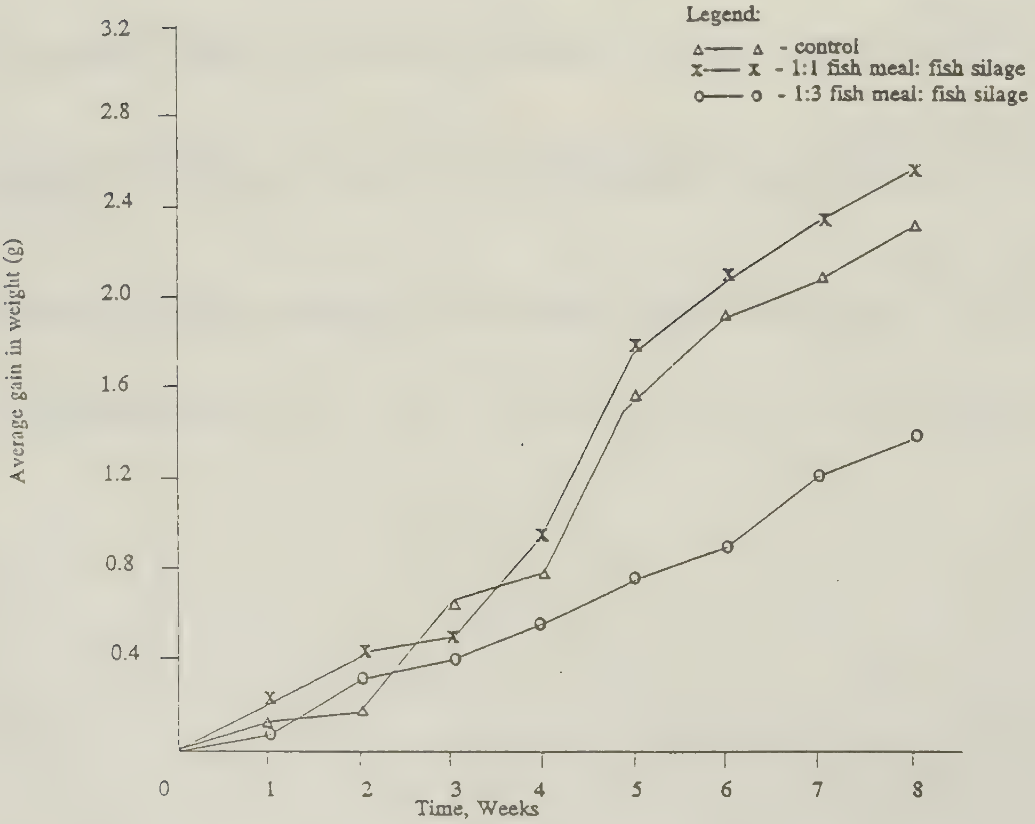

Figure 2. Average gain in weight of fish in 8 weeks feeding (Trial II)

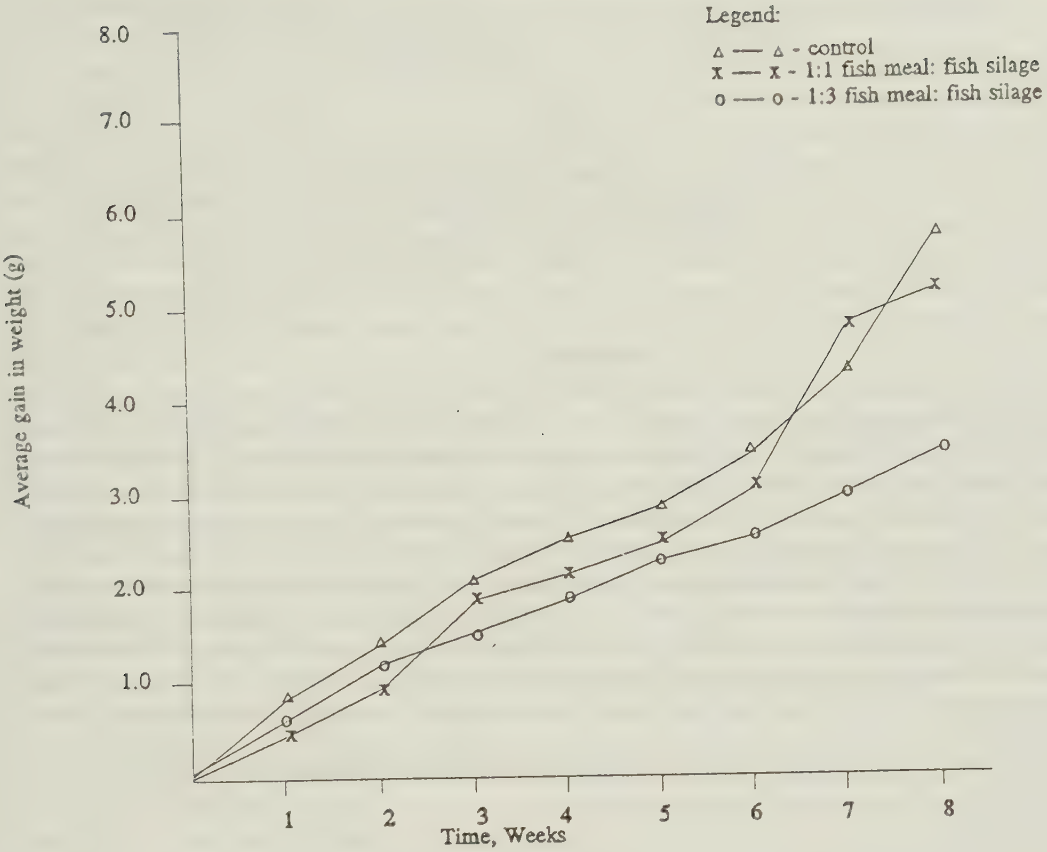

Figure 3. Average gain in weight of fish in 8 weeks feeding (Trial III)

THE IMPORTANCE OF *LISTERIA MONOCYTOGENES* IN PROCESSING AND MARKETING OF SEAFOODS

by

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ABSTRACT

The importance of *Listeria monocytogenes* in seafoods traded in international markets has come to prominence over the past five years. Importing countries, in particular the United States, require that *L. monocytogenes* be absent in ready-to-eat foods, which include cooked and smoked seafoods. There is little information on the role of seafoods in the transmission of listeriosis, although the organism has been isolated from a variety of processed and raw fishery products. Data from the Natural Resources Institute suggest that raw and partially cooked, chilled products which are consumed without further processing should be classified as high risk foods. Traditional processed seafoods, such as fish pastes and sauces, and dried and hot smoked (cooked/dried) products are unlikely to be of importance in the spread of disease.

INTRODUCTION

For many countries in the tropics exported seafoods provide an important source of revenue. The exported goods, however, must meet standards set by agencies in the importing countries. Amongst the criteria to be met are recommended levels for bacteria such as *Salmonella* and *Vibrio cholera*. In recent years standards have also been set for *Listeria monocytogenes* in seafoods.

L. monocytogenes was first recognised to be an important foodborne pathogen in the 1980's. This followed several large outbreaks of listeriosis in North America and Europe in which there was strong evidence that food was the source of infection (Schlech *et al*, 1983, Fleming *et al.*, 1985; Linnan *et al.*, 1985; Billie and Glauser, 1988). Although the disease is relatively rare and only affects certain groups within the population, namely pregnant women, foetuses and immuno-compromised individuals, it is considered to be serious because of the high mortality rate (approximately 30%).

Many seafoods are exported raw and frozen, but there is a growing demand for added value products such as cooked seafoods. The emergence of *L. monocytogenes* as a problem has, however, created challenges for food processors who must now ensure that any foods which are to be consumed with only minimal or no cooking, are free of the organism. The United States Food and Drug Administration (FDA) monitors imports, and requires that *L. monocytogenes* is absent in 25g samples of ready-to-eat foods, which include cooked and smoked seafoods. The US National Advisory Committee on Microbiological for Foods has recommended microbiological criteria for cooked ready-to-eat shrimp and crabmeat. These state that *L. monocytogenes* should not be present in a 25g sample taken from a composite of 5 units of 50g (Buchanan, 1991).

The characteristics of *L. monocytogenes* differ from those of other common foodborne pathogens. The one of greatest importance to the food industry is the fact that it can grow at refrigeration temperatures previously considered to be adequate in preventing the growth of pathogenic bacteria in foods. *L. monocytogenes* can survive for considerable lengths of time in frozen products, it can grow in relatively high salt concentrations, and can also survive in the presence of certain preservatives. This paper will describe the incidence of the organism in seafoods and look at the implications that this has for the processors, with particular reference to developing countries.

OCCURRENCE IN SEAFOODS

L. monocytogenes was first reported to cause disease in humans by Nyfeldt in 1929 (Gray and Killinger, 1966), but it was only recently that the role of food in the transmission of the organism was established. A number of well documented outbreaks of listeriosis occurred in which dairy and vegetable products were implicated as the sources of infection. Research into the organism subsequently concentrated on these commodities. In 1980 there was an outbreak of disease in Auckland, New Zealand, involving 22 perinatal cases and resulting in 5 deaths. Investigators suggested that the consumption of raw seafood may have been a contributory factor, although the evidence presented was epidemiological rather microbiological (Lennon *et al., 1984* Certainly, *Listeria* species including *L. monocytogenes* are known to occur in seafoods. Gray and Killinger (1966) isolated *Listeria* spp. from rainbow trout. Fuchs & Surendran (1989) examined fresh fish in India and found that 30% of the samples contained *Listeria innocua,* a species considered to be non pathogenic; no *L. monocytogenes* was isolated. One out of 35 oyster samples obtained from the Californian coast was found to contain *L. innocua* (Colburn *et al.,* 1990). Buchanan *et al.* (1989) isolated *L. monocytogenes* from 15% of uncooked seafood samples examined. One sample of raw minced fish (coalfish) out of 8 samples examined was found to contain *L. monocytogenes* (Rorvik and Yndestad, 1991). These findings suggested that seafoods could act as a vehicle for transmission of *Listeria* to humans.

There are a number of different routes by which *Listeria* can enter water and thereby contaminate fish, the main ones being run-off from agricultural lands, direct faecal contamination by animals, and release of sewage effluent. Raw animal slurry may be sprayed onto agricultural land, where the organism can survive for long periods of time. Watkins and Sleath (1981) found that numbers of *L. monocytogenes* in sewage sludge spread onto land remained unaltered over an 8 week period. Colburn *et al* (1990) examined fresh river water and sediment in California and found *Listeria* spp. in 81% of the water samples and 30% of the sediment samples. The predominant species in the sediment was *L. monocytogenes*. Specific *Listeria* species or *L. monocytogenes* serotypes were found to predominate in the water when animals were being kept in close proximity to the sample sites. There was a greater variety in the species, with no one showing predominance, in areas where there were no farm animals. Watkins and Sleath (1981) also found *Listeria* spp. in all of 7 river water samples taken in the United Kingdom. The majority of isolates were found to be haemolytic.

Sewage and processing effluents are sources of *Listeria* in aquatic environments. Al-Ghazali and Al-Azawi (1986) isolated *L. monocytogenes* at various stages of sewage treatment, including the final effluent. Similar findings have been made by Motes (1991) and Watkins and Sleath (1981), the latter also isolating *L. monocytogenes* from processing plant effluent.

These findings suggest that fresh water tributaries, together with sewage and processing effluents, may introduce *Listeria* into the marine environment. In addition, it has been shown that birds, and in particular gulls, are carriers of *Listeria* and may be a source of the organism (Fenlon, 1985). What was not clear until recently was whether *Listeria* could survive in the marine environment. It is known that *Listeria* can grow in 10% salt solutions (Seeliger, 1961) and can survive at higher concentrations (Shahamat *et al*, 1980). Recently, Colburn *et al* (1990) suggested that this could be partly due to the dilution of organisms on entering the marine environment. One study has also shown that when low levels of *L. monocytogenes* are inoculated into seawater, numbers of the organism decrease (Faud *et al*, 1989).

FROZEN FISHERY PRODUCTS

Raw seafoods are often frozen for storage and distribution, but several studies involving other foods have shown that *L. monocytogenes* can survive under these conditions. Slivko (1958) inoculated mutton with *Listeria* and isolated the organism after storage at -10°C and -23°C for up to 20 days. Lehnert (1960) reported that *L. monocytogenes* survived for two years at -20°C after 35 thawings. Numbers of *L. monocytogenes* inoculated into butter and stored at -18°C for 70 days were not appreciably reduced (Olsen *et al*, 1988). If the organism is present in seafoods, freezing may, therefore, not significantly affect numbers.

Weagant *et al* (1988) examined frozen raw seafoods being imported into the USA and found *L. innocua* in 44% of the samples, and *L. monocytogenes* in 24%. Fuchs and Surendran (1989) isolated *L. innocua* from 36% of frozen raw fish samples tested. A survey carried out in Taiwan by Wong *et al* (1990) found 10.5%

of frozen seafood samples to contain *L. monocytogenes*. *Listeria* spp. were isolated from 49% of raw frozen seafood samples tested by Noah *et al* (1991). Motes (1991) isolated *L. monocytogenes* from 11% of frozen raw shrimp samples.

Although *L. monocytogenes* does occur in raw and frozen raw seafoods, these products do not pose a great health threat to the majority of people. On the whole seafoods undergo some further processing before being eaten, and it is the processed products which are to be consumed without further cooking which are of greatest concern. However, there are groups of people around the world who eat seafoods raw, and for these people there may be a risk attached to eating uncooked fish or seafoods.

QUALITY CONTROL IN SEAFOOD PROCESSING PLANTS

Care must be taken in seafood processing environments as *L. monocytogenes* may be brought into the factory on the raw product. Being an environmental organism, *L. monocytogenes* can also be introduced into processing areas by a number of different routes. In order to obtain more information about the incidence of *Listeria* in a factory environment, Fuchs (unpublished date) carried out a survey in shrimp processing plants of one major SE Asian seafood exporting country. *L. innocua* was isolated from various locations in the plants, including containers, trays, processing tables, weighing equipment, floors, drains and workers' boots. This species of *Listeria* is more frequently isolated from the environment that *L. monocytogenes,* but it is possible that the pathogen may also be present. These findings are similar to those reported by Cox *et al.* (1989) for food processing factories.

Micro-organisms in the processing environment can be controlled through the use of sanitising agents. Studies have been carried out on the effects of chlorine on *L. monocytogenes*. Chlorine is still the most commonly used sanitising agent in developing countries. El-Kest and Marth (1988a) found that chlorine was almost as effective at inactivating *L. monocytogenes* as it is for the other non-spore forming bacteria. Exposure of cells to 0.5, 1, 2, 5 and 10 ppm available chlorine resulted in D-values of 61.7, 11.3, 6.7, 4.9 and 4.7 secs respectively during the first 30 secs of exposure. The same authors looked at the effects of strain variation, temperature and pH on inactivation of *L. monocytogenes* by chlorine (El-Kest and Marth, 1988b). They found that sensitivity to chlorine varied from strain to strain and that increasing pH reduced the efficiency of chlorine. Chlorine solutions were also found to be more effective against *Listeria* at 5°and 35° than at 25°. These findings imply that the use of permitted levels of chlorine on food contact surfaces (200 ppm) are sufficient to destroy the organism. However, Archer (1990) suggested that *Listeria* may be resistant to sanitisers in food processing environments if they are able to form biofilms. Lee and Frank (1991) determined the inactivation by hypochlorite of *L. monocytogenes* cells adhered to stainless steel. They found that cells allowed to grow on the surface for 8 days were over 100 times more resistant than cells grown for 4 h when exposed to 200 ppm hypochlorite for 30 secs. Mustapha and Liewen (1989) also reported that a 5 minute exposure to 200 ppm chlorine solution is required to destroy *L. monocytogenes* on contaminated stainless steel surfaces. They found that cells allowed to grow on the surface for 8 days were over 100 times more resistant than cells grown for 4 h when exposed to 200 ppm hypochlorite for 30 secs. Mustapha & Liewen (1989) also reported that a 5 min exposure to 200 ppm chlorine solution is required to destroy *L. monocytogenes* on contaminated stainless steel surfaces.

The data led to the conclusion that frequent cleaning and the use of sanitisers must be employed as part of an effective cleaning programme. Seafood processing plants, particularly those in the tropics, are ideal environments for the survival and growth of *Listeria,* being both wet and warm. Producers of processed seafood products must therefore be aware that there is a risk of post-process contamination if the environment is not kept adequately clean and sanitised, and well segregated from areas where raw product is handled.

A great deal of work has gone into developing methods for isolating *L. monocytogenes* from foods, although little has been done to assess their application to seafoods. Two commonly used isolation procedures are those recommended by the FDA (Lovett *et al.*, 1987) and the US Department of Agriculture (USDA, McClain and Lee, 1988). Lovett *et al.* (1991) quantitatively compared the two procedures for their ability to isolate *L. monocytogenes* from seafoods. They found that the FDA method isolated heated *L. monocytogenes* from seafoods at a lower level than the USDA method and was therefore probably better for processed products. For isolating *Listeria* from raw seafood and for recovering non-heat stressed *Listeria* the

USDA method gave better results. The greater selectivity of plating media used in the USDA method may explain the lower recovery of heat stressed cells. Noah *et al.* (1991) compared four different selective enrichment procedures for the recovery of *Listeria* from seafoods using an ELISA procedure to detect the organism after enrichment. They recommended the FDA enrichment broth as a suitable broth to use in the recovery of *L. monocytogenes* from seafoods. Ten selective direct plating media for the enumeration of *L. monocytogenes* inoculated into raw oysters were compared by Cassiday *et al.* (1989). They selected Dominguez Rodriguez Isolation Agar as the most suitable medium, basing the decision on relative recovery of viable cells, as well as ease of recognising and enumerating *L. monocytogenes* colonies.

Since the study conducted by Cassiday *et al.* (1989) took place, some new diagnostic media, such as Oxford medium (Curtis *et al.,* 1989) and PALCAM (van Netten *et al.,* 1989), have become available. These media allow diagnosis of *Listeria* by utilising the fact that all the species hydrolyse aesculin. Inclusion of a ferric salt in the medium leads to the formation of a black complex. In addition, PALCAM contains mannitol and phenol red. *L. monocytogenes* does not ferment mannitol and can easily be differentiated from those organisms which hydrolyse aesculin and also ferment mannitol, thereby giving rise to a change of colour in the medium. These media are much easier to use than some of the older media such as Modified McBride Agar and Lithium chloride- phenylalanine-moxalactam Agar on which colonies have to be observed using a transillumination technique first described by Henry (1933). The method currently used at NRI for the isolation of *L. monocytogenes* from seafoods is outlined in Figure 1 (Fuchs, 1991). it includes a non-selective pre-enrichment stage to aid the resuscitation of injured cells from processed products. The USDA two stage selective enrichment procedure (UVM I and UVM II) is then used (McClain and Lee, 1988), followed by plating onto Oxford medium (Curtis *et al.*, 1989) or Modified Oxford medium (McClain and Lee, 1989).

Figure 1. Procedure for the isolation of *Listeria* from foods (Fuchs, 1991)

25g sample + 225 ml pre-enrichment broth
(PEB - Buffered Peptone Water or Trypticase
Soy Broth with 0.6% yeast extract)

↓ 24h at 30°C

10 ml PEB + 90 ml Uvm I

↓ 24h at 30°C

0.1 ml UVM I + 10 ml Uvm II

↓ 24h at 30°C

Streak UVM II onto Oxford medium
or Modified Oxford medium

↓ 24/48h at 35°C

Examine plates for typical colonies
and pick for characterisation

Following isolation of the organism, further information may be obtained by typing the isolates. *L. monocytogenes* can be divided into at least 13 different serotypes, although the majority of isolates from clinical cases are 4b, 1/2a and 1/2b (McLaughlin, 1987). There have been few studies of the distribution of serotypes in seafoods. Weagant *et al.* (1988) serotyped *L. monocytogenes* isolates from frozen seafoods and found 4b to be the predominant typable serotype. Wong *et al.* (1990) serotyped *L. monocytogenes* isolated from a number of different foods and found 74% serotype 1 and 26% serotype 4 in seafoods; no other serotypes were isolated. They compared these with isolates from pork and chicken, the majority of which were other serotypes. Jemmi (1990) also reported that the most common serotypes isolated from smoked and brined fish were 1/2a (20%), 1/2b (59%) and 4b (14%). Rorvik & Yndestad (1991) found that all isolates of *L. monocytogenes* from seafoods were either 1 (43%) or 4 (57%). Typing systems based on antisera raised against *Listeria* provide little information for epidemiological studies, as the majority of isolates fall within the 3 groups mentioned. Work is under way to develop other typing systems, such as phage typing (Audurier and Martin, 1989) and isoenzyme analysis (Bibb et al., 1989), in order to show that isolates obtained from foods and listeriosis patients are the same. These methods have not yet been used to examine isolates obtained from seafoods.

PROCESSED SEAFOODS

In some countries seafood is eaten with only a minimal amount of processing. A popular dish in South America *(ceviche)* and on many Pacific islands is raw fish marinated in lime or lemon juice. The dish is prepared by slicing raw fish or molluscs and marinating them in the citrus juice for at least one hour prior to consumption. Seasoning, celery, onions and hot chili peppers can also be added. Similar products exist in SE Asia where nipa palm vinegar is used in addition to lime juice, and the products can be stored at refrigeration temperatures for up to 8 hours before consumption. It has been demonstrated in a number of food systems studied that *Listeria* are not able to grow at pH values lower than 4.8 - 5.0, the organism favouring a neutral to slightly alkaline pH (Conner et al., 1986; Parish and Higgins, 1989). The lowest pH at which growth has been recorded in a non-food system is 4.39 in trypticase soy broth adjusted with HCl and inoculated at 30°C (George et al., 1988). Although growth may not occur, L. *monocytogenes* can survive under acidic conditions. In orange serum adjusted to pH 3.6 with HCl and inoculated with 2.2 x 100 cfu/ml *L. monocytogenes,* numbers of viable cells had only decreased by approximately one order of magnitude after 2 days at 30°C (Parish and Higgins, 1989). Fuchs & Sirvas (1991) examined 32 samples of ceviche purchased from market stalls around Lima, Peru. *L. innocua* was isolated from 75% of the samples, and *L. monocytogenes* was found in 9% of the samples tested. The pH of the marinades ranged from 3.8 to 4.8, some buffering being given by the fish flesh. The pH of the fish flesh in ceviche ranged from 4.2 to 5.1. The lowest pH of fish meat from which *Listeria* were isolated was 4.4. This product has recently attracted attention after it was reported that it may have played a role in the spread of *Vibrio cholerae,* thereby giving rise to the present epidemic of cholera in South America.

Fermented fishery products and fish pastes are popular products in SE Asia. Few data are available on their potential to support the growth of *L. monocytogenes.* Some of these products such as Philippine patis and bagoong, Thai nam-pla and Vietnamese nuoc-mam have salt concentrations as high as 30%. High salt concentrations will prevent the growth of many microorganisms, but *L. monocytogenes* can grow in concentrations up to 10% (Seeliger, 1961), and can survive in concentrations up to 30% (Shahamat *et al.,* 1980). These traditional products are unlikely to support the growth of *L. monocytogenes.* Other fermented products, such as the lactic fermented seafoods *(buros* of the Philippines and *pla-som-fak* in Thailand) rely on a combination of salt (2-12%) and lowered pH (4-5) for preservation. The combined effects of salt and low pH, together with possible antimicrobials produced by lactic acid bacteria should minimise the potential for growth of L. *monocytogenes* in these products. Some lactic acid bacteria have been shown to produce bacteriocins which are inhibitory to *L. monocytogenes* (Harris *et al.,* 1989).

Some workers have examined salted seafoods for the incidence of *L. monocytogenes.* Jemmi (1990) and Hartemink & Georgsson (1991) tested low salt (2-9%) gravad fish and both isolated *L. monocytogenes* from 26% of the samples. Rorvik & Yndestad (1991) isolated *L. monocytogenes* from 18% of brined shrimp samples examined. Salting can also be combined with drying as a method of preserving fish. Fuchs & Surendran (1989) examined 11 samples of dried, salted fish in India. The samples had a moisture content of 20-25% and a salt content of 15-20%. When these products are prepared they are first salted and then sun-dried for 2 days. *Listeria* were not isolated from any of the samples, although during drying there is a risk of faecal contamination from birds.

A large proportion of crustaceans is cooked before being sold to the consumer. The heat resistance of *L. monocytogenes* in chicken, beef steak and carrot homogenates was determined by Gaze *et al.* (1989). At 70°C the D-values ranged from 0.14 to 0.27 min. The authors recommended that in order to achieve a

6 log reduction of the organism, the slowest heating point in a food product should be held at 70°C for 2 min. *L. monocytogenes* is therefore not a particularly heat tolerant organism and most commercial cooking processes are adequate to destroy the organism. Nevertheless, *L. monocytogenes* has been isolated from cooked seafoods. Weagant *et al.* (1988) reported that 47% of cooked frozen samples contained *L. innocua,* and *L. monocytogenes* was isolated from 28% of the samples. Noah *et al.* (1991) found *Listeria* spp. in 20% of samples of frozen or chilled cooked crabmeat. The results suggest that either the heat treatment had been inadequate to destroy the organism, or that post-processing contamination had occurred.

Another common processing method used to preserve fish is smoking. The preserving effect results from the combination of drying and deposition of chemicals present in the smoke produced from burning wood. Smoking can either be carried out at a relatively low temperature, in which case the main effects of smoking are to deposit flavour and preservative compounds onto the fish, or at a higher temperature leading to cooking or partial cooking and drying of the product. Cold smoking of fish from temperate climates is usually carried out at temperatures of less than 30°C, whereas the temperatures for tropical fish range from 30° to 40°C. Temperate water fish are hot smoked at temperatures of approximately 85°C, whilst the temperatures for tropical fish are in excess of 100°C. These products are also generally salted prior to smoking. Some smoked products in SE Asia are cooked in a saturated brine solution prior to a short hot smoking period and have a limited shelf-life at ambient tropical temperatures. In West Africa smoked fish can be fully cooked and dried, and in some instances re-smoked. The final, dry product is unlikely to support the growth of *L. monocytogenes.*

Hartemink & Georgsson (1991) isolated *L. innocua* from 29% and *L. monocytogenes* from 6% of smoked fish samples. The fish had been smoked at a relatively low temperature, although the manufacturers had not provided exact details of the process. Rorvik & Yndestad (1991) isolated *L. monocytogenes* from 9% of 33 smoked salmon samples analyzed. Jemmi (1990) examined both cold and hot smoked fish and isolated *L. monocytogenes* from 14% of cold smoked and 9% of hot smoked samples. Guyer & Jemmi (1990) examined smoked salmon, a cold smoked product, and isolated *Listeria* spp. from 14% of the samples, *L. monocytogenes* from 7%, *L. seeligeri* from 5.5% and *L. innocua* from 2 %. The same workers went on to measure levels of *L. monocytogenes* on inoculated salmon fillets which were marinated, cold-smoked and stored at 4° or 10°C for up to 30 days (Guyer and Jemmi, 1991). The marination and smoking stages did not greatly affect the numbers of *L. monocytogenes* present on the fish, although in samples with an initial inoculation level of 10^2 MPN/g and above, significant growth was measured during storage. These worker's results suggest that smoking fish does not inactivate *Listeria.* Contrary to this, initial studies at the Natural Resources Institute (NRI) suggest that *L. monocytogenes* inoculated onto agar plates is rapidly killed off in wood smoke at 30°C. The fact that L. *monocytogenes* can occur in smoked fish led the British Government to consider banning the sale of smoked salmon by mail order companies, but they have now decided against this.

Once seafoods have been cooked or smoked they are often stored at refrigeration temperatures in retail outlets. If L. *monocytogenes* is present in the product after processing, there is the potential for it to grow to high levels if held at these temperatures for a considerable length of time. Some seafood products may be stored under these conditions for 2 weeks or more. *Listeria* can grow at 1°C with some strains capable of growth at temperatures as low as 0.5°C (Juntilla *et al.,* 1988). The growth of *L. monocytogenes* at low temperatures has been demonstrated in a number of foods including milk (Donnelly and Briggs, 1986), butter (Olsen *et al.,* 1988) and lettuce (Beuchat and Brackett, 1990). Brackett & Beuchat (1990) measured the growth of *L. monocytogenes* in crabmeat stored at 5° and 10°C and found that with an inoculum of approximately 10^4 cfu/g, numbers reached 4.9×10^6 cfu/g after 14 days and in excess of 10^8 cfu/g after 8 days at each of the respective temperatures. This suggests that if *L. monocytogenes* is present in cooked seafoods, even in low numbers, quite high levels may be reached if the products are stored at temperatures commonly encountered in the chill cabinets of retail outlets. Numbers may further increase during storage by the consumer. The infectious dose of *L. monocytogenes* in humans has not been determined. Studies using immunocompetent mice have shown that levels of L. *monocytogenes* as high as 2.5×10^8 cfu/g may be required to bring about infection, if administered orally. If the mice are immunosuppressed this figure can be significantly reduced.

Several papers have also reported that the virulence of L. *monocytogenes* increases when the organism is grown at low temperatures (Gray and Killinger, 1966; Wood and Woodbine, 1979; Brasher *et al.,* (1984).

Brackett & Beuchat (1990) investigated the pathogenicity of *L. monocytogenes* grown on crabmeat and could find no difference in cultures grown at 5°C and 10°C.

CONCLUSIONS

Our review demonstrates that there are public health risks associated with *L. monocytogenes* in seafoods. Raw or minimally processed products belong to a high risk group. Of concern also are products which are stored at refrigeration temperatures and which have undergone processing not sufficient to kill *L. monocytogenes,* or which are at risk of postprocessing contamination. Traditionally processed tropical seafoods, such as fish pastes and sauces, lactic fermented products, dried salted and hot smoked (cooked/dried) products are unlikely to be of importance in the spread of listeriosis.

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INCIDENCE AND VIABILITY OF *V. CHOLERAE* IN SEAFOOD HANDLING AND PROCESSING

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ABSTRACT

Although the incidence of *V. cholerae* in frozen seafoods produced in the southern part of India is infrequent, the organism survives freezing and further storage at -18°C for up to 142 days. In water from the public supply *V. cholerae* survives for 35 to 40 hours at 30°C and for 5 to 10 days at refrigerated temperatures (5 to 10°C). The water used in the seafood processing factories should be chlorinated to a residual level of 10 ppm to get full protection against *V. cholerae.*

INTRODUCTION

Seafoods have often been reported to be contaminated with *Vibrio cholerae* (de Paola 1982, De *et al*, 1977 and Varma *et al,* 1989). For the past few years Japan has been the major buyer of Indian marine products particularly shrimps. However, a few consignments of frozen shrimps exported from India suffered rejection in Japan due to the detection of *Vibrio cholera*. In this context, detailed studies were carried out at the Central Institute of Fisheries Technology, Cochin, on the incidence of *V. cholerae* in seafoods and its viability during freezing and frozen storage. As earlier studies conducted at the institute revealed water as one of the major sources of contamination (Iyer *et al.* 1988a), it was considered essential to study the viability of the organism in the water during storage and during chlorination. The results obtained in the above studies are presented in this paper.

MATERIALS AND METHODS

The samples of the marine products were drawn from different seafood processing units, primary process centres, landing places and fish markets situated along the coasts of the states of Kerala and Tamil Nadu in South India, which together contribute about 48% of the total marine products exported from India. The samples were collected aseptically and brought to the testing laboratory in ice boxes. In all cases the analysis was started within 30 minutes of the sample collection.

To study the viability of the organism in shrimp during freezing and frozen storage, samples of fresh shrimp - both headless and shell-on and peeled and deveined - were inoculated with pure strains of *Vibrio cholerae* Inaba and Ogawa and were frozen and stored at -18°C. The samples were withdrawn periodically and tested for the organism.

For the study on the viability of *V. cholera* in water, samples of water taken in glass containers of 5 l capacity were inoculated with pure known strains of *V. cholera* and were stored at ambient temperatures (28-30°C) and at refrigerated temperatures (5-10°C). The samples were tested periodically for the organism.

To study the effect of chlorination on the viability of the organism in water, the water samples inoculated with pure known cultures of *V. cholerae* were chlorinated to 5, 10 and 15 ppm chlorine levels with a contact time of 3, 5 and 10 minutes and the samples were subsequently tested for the organism.

In all the above studies, the *V. cholerae* was detected and confirmed by the method described by Iyer *et al.* (1988a) and USFDA (1978).

RESULTS AND DISCUSSION

Details of samples collected and the incidence of *V. cholerae* 01 and non-01 are given in Table 1. The table indicates that *V. cholerae* 01 was absent in all the frozen samples tested. However, this organism was detected from 2 samples (0.2%) of fresh seafoods. The first one isolated from fresh shrimp on serotyping was found to be "Ogawa" and the second one, isolated from fresh cuttlefish fillets "Inaba". Non-isolation of *V. cholerae* 01 from frozen samples may be due to the better hygienic and sanitary conditions prevailing in the processing units compared to the pre-processing units and markets. Incidence of *V. cholerae* non-01 was noted in all categories of samples except frozen cuttlefish. This has got some significance because there are some references showing the pathogenicity of this organism (Aldova *et al* 1968; CDC, 1969; De Paola, 1981). As seen from the Table, there was a marked variation in the percentage incidence of *V. cholerae* non-01 between unfrozen and frozen samples and also between different categories of products. In all cases significantly low levels of incidence can be seen in frozen samples compared to the corresponding unfrozen samples. Among the raw fish, fin fish showed the highest percentage (37.6) of incidence of *V. cholerae* non-01. De *et al.* (1977) have also reported a similar observation (38%) on the incidence of *V. cholerae* non-01 in marine fish samples collected from the Calcutta region.

Table 1 Incidence of *V. cholerae* 01 and non-01 in fish

Samples	No. of samples examined		No. of samples showing incidence of *V. cholerae*-01		No. of samples showing incidence of *V. cholerae* non-01	
	Raw	Frozen	Raw	Frozen	Raw	Frozen
Shrimp	728	287	1 (0.14)	0	194 (26.6)	34 (11.8)
Cuttle fish	23	4	1 (4.3)	0	7 (30.4)	0
Squid	46	44	0	0	6 (13.0)	5 (11.3)
Lobster	13	160	0	0	3 (23.0)	30 (18.7)
Clam	31	95	0	0	11 (35.4)	17 (17.8)
Finfish	85	143	0	0	32 (37.6)	3 (2.0)
Mussel	75	-	9	0	10 (13.3)	-
Total	1001	733	2 (0.2)	0	263 (26.3)	89 (12.14)

Figures in parenthesis are the percentages.

Table 2 gives the viability of *Vibrio cholerae* in shrimp during freezing and frozen storage. It is seen from the Table that the organism survives freezing and further storage at -18°C for up to 142 days. There is no difference in the viability of the two different serotypes of the organism, Ogawa and Inaba, in shrimp during freezing and subsequent frozen storage. However, the viability was slightly more in headless shell on shrimp compared to that in peeled and deveined shrimp.

Table 2 Viability of *Vibrio cholerae* during freezing and frozen storage

No. of days of frozen storage	Peeled and deveined shrimp			Headless shell on shrimp		
	Ogawa	Inaba	Non-01	Ogawa	Inaba	Non-01
Immediately after freezing	+	+	+	+	+	+
10	+	+	+	+	+	+
16	+	+	+	+	+	+
23	+	+	+	+	+	+
45	+	+	+	+	+	+
60	+	+	+	+	+	+
72	+	+	+	+	+	+
88	+	+	+	+	+	+
101	+	+	+	+	+	+
131	+	+	+	+	+	+
142	-	-	-	+	+	+
151	-	-	-	-	-	-
160	-	-	-	-	-	-

+ = Organism present
- = Organism absent

Viability of *Vibrio cholerae* in water collected from three different sources are indicated in Table 3. In distilled water at 30°C, *V. cholerae* survived only for 17-20 hours, whereas at refrigerated temperatures the organism survived up to 5 days. In the public supply water, *V. cholerae* 01 survived for 35 to 40 hours at 30°C and for 5-10 days at refrigerated temperatures. Sterilization of the public supply water before inoculation of the organism extended the survival period up to 2 to 3 days and 10-16 days at ambient temperatures and refrigerated temperatures respectively. The organism was viable in sea water for 9 to 10 days at 30°C and 60-70 days at refrigerated temperatures. Again, sterilization of sea water before inoculation extended the viability to 38-40 days at 30°C and 165 to 170 days at refrigerated temperatures.

Table 3 Viability of *V. cholerae* in different sources of water

Source of water	El Tor Inaba		El Tor Ogawa	
	Room temp.	Refrigerated temp.	Room temp.	Refrigerated temp.
* Distilled water	17-20 hrs.	5 days	17 hrs.	5 days
** Municipal water	35-40 hrs.	5-9 days	35-40 hrs.	5-10 days
Sterile municipal water	2-3 days	10-16 days	2-3 days	10-16 days
*** Sea water	9-10 days	60-70 days	4-5 days	45-50 days
Sterile sea water	38-40 days	165-170 days	32-35 days	150-160 days

pH	NaCl %
* 5.63	0
** 7.1	0.001
*** 7.7	1.7

The viability of *Vibrio cholerae* in water subjected to different levels of chlorination is summarised in Table 4. From the results, it is clear that chlorination of process water to a residual level of 5 ppm is not sufficient to destroy viable cells of *V. cholerae*. Similarly, it is also evident that a contact time of 3 minutes is not sufficient to get full protection against *V. cholerae*. If the concentration of chlorine used for disinfection is 10 ppm even a contact time of 3 minutes becomes insufficient when the number of *V. cholerae* cells exceeds 70,000/ml.

Table 4 Viability of *V. cholerae* in water during chlorination

Treatment time	Cells	*V. cholerae* ogawa			*V. cholerae* inaba			*V. cholerae* non-01		
		Growth after exposure to different chlorine levels								
		5 ppm	10 ppm	15 ppm	5 ppm	10 ppm	15 ppm	5 ppm	10 ppm	15 ppm
3 mins.	1,500	+	-	-	+	-	-	+	-	-
5 mins.		+	-	-	+	-	-	+	-	-
10 mins.		-	-	-	-	-	-	-	-	-
3 mins.	3,500	+	-	-	+	-	-	+	-	-
5 mins.		+	-	-	+	-	-	+	-	-
10 mins.		-	-	-	-	-	-	-	-	-
3 mins.	6,500	+	-	-	+	-	-	+	-	-
5 mins.		+	-	-	+	-	-	+	-	-
10 mins.		-	-	-	-	-	-	-	-	-
3 mins.	18,000	+	-	-	+	-	-	+	-	-
5 mins.		+	-	-	+	-	-	+	-	-
10 mins.		-	-	-	-	-	-	-	-	-
3 mins.	70,000	+	+	-	+	+	-	+	+	-
5 mins.		+	-	-	+	-	-	+	-	-
10 mins.		+	-	-	-	-	-	-	-	-
3 mins.	108,000	+	+	-	+	+	-	+	+	-
5 mins.		+	-	-	+	-	-	+	-	-
10 mins.		+	-	-	-	-	-	-	-	-

+ = Growth present
- = Growth absent

The studies in general indicate that the incidence of *Vibrio cholerae* in seafoods collected from the southern part of India is insignificant. The organism survives in shrimp during freezing and subsequent storage for about 5 months at -18°C. It is viable for longer period in water at refrigerated temperatures. The seafood processors are advised to chlorinate the process water to a residual level of 10 ppm to get full protection against *V. cholerae*.

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PRESERVATION PROCESSES FOR READY-TO-COOK/SERVE FISH AND SHELL FISH PRODUCTS AT TROPICAL TEMPERATURES

by

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ABSTRACT

The composition of a modified preservative-condiment mixture for storage of fresh slices at ambient temperature and the results of storage studies of the treated samples of six species of fish are reported. The formulation contains calcium propionate, sorbic acid, tartaric acid, sodium chloride, calla powder and turmeric powder. Portions from six species of fish treated with the preservative mixture could be stored in edible conditions from one to three days at ambient temperature (28°C to 32°C) depending upon species and freshness. Composition of a preservative mixture for ready-to-serve fried mussel meat for storage at room temperature and the results of storage studies are also reported. There was an inverse relationship between moisture and shelf life. Fried mussel meat with a moisture content of around 10% remained in edible condition for five months. Fried mussel meat treated with condiments alone remained in good condition for 4, 5 and 45 days when moisture contents of the samples were 40%, 30% and 15% respectively compared to 8, 12 and >60 days when preservatives were also added.

INTRODUCTION

Fresh fish and shellfish become inedible at tropical temperatures within less than twelve hours. Though icing extends the shelf life to two weeks it causes loss of nutrients and flavour bearing components. Higher transportation cost due to the increased bulk and weight of added ice and the reicing steps during storage are additional disadvantages. A process capable of extending shelf life at ambient temperature would be advantageous in the efficient distribution of fish for human consumption. The limitations of waste disposal facilities in city dwellings and on the available time of employed housewives necessitate production of ready-to-cook/serve fish preparations. Nair *et al.* (1990) reported a preservative formulation for storage of fresh fish slices at room temperature for one to four days in edible condition. However, further consumer evaluation of such treated products revealed that the product after frying was too salty. This communication reports the composition of a modified preservative-condiment mixture for storage of fresh fish slices at ambient temperature as well as the storage characteristics of different species of treated fish slices. Also included in this report is a process for preparation of fried mussel meat and its shelf life at tropical temperatures.

MATERIALS AND METHODS

All the storage studies were carried out in the laboratory at 28-32°C.

Ready-to-cook fish slices

Reasonably fresh marine and brackish water fish available in the local fish landing centres and markets were used in these experiments. Larger fish were scaled, gutted, headed and cut into slices of about 10 mm thickness. Small fish were scored in the flesh instead of slicing. They were washed in potable water and drained. Calcium propionate, sorbic acid and tartaric acid were used as preservatives. Common salt, calla powder and turmeric powder were the other ingredients used. To determine the optimum level of these preservatives and ingredients, different experiments were carried out with mixtures of the components at different levels. Each mixture was tested for its ability to extend shelf life at ambient temperature by mixing the dry mixture with the fish slices, packing in 200 gauge polythene bags, storing in the laboratory and observing for quality deterioration every day by sensory characteristics. After thorough sensory evaluation, in the raw state during storage and after drying, a formula was arrived at for room temperature preservation of fresh fish slices. Portions of tuna, black kingfish, and drab jew fish were tested for bacteriological characteristics, chemical indices of spoilage (like total volatile nitrogen (TVN) and trimethylamine nitrogen (TMAN) by standard procedures in addition to sensory evaluation. A limited number of consumers were supplied with treated fish slices immediately after treatment and at daily intervals up to three days to get their opinion on the acceptability of the samples.

Ready-to-serve fried mussel meat

Green mussels (*Perna viridis*) were used in these experiments. The live mussels were brought to the laboratory and the dirt and debris on the shell were washed off. They were then heated in a vessel till the shells opened and the meat was shucked. The shucked meat was dressed, washed and drained. In the initial experiments frying was done in a limited quantity of coconut oil after mixing with condiments as is practised in households or with condiments and the preservatives. However, this method of frying resulted in a product with poor appearance and with oil sticking on to the mussel meat. Hence, further experiments were carried out by deep fat frying of the washed and drained meat. This helped to retain a better appearance and the product was free of adhering oil. Further, the oil was clear and could be used for fdrying additional batches of mussels. To study the effect of moisture on shelf life, mussel meat was fried to three different moisture levels and they were mixed with the condiments or condiments and preservatives fried in a small quantity of oil. Condiments were added as a percentage of the fried meat. They were packed in 200 gauge polythene bags, stored in the laboratory and observed for sensory characteristics and growth of fungi. In the final experiments the drained mussel meat was deep fat fried to nearly 10% moisture, mixed with the formulation of salt, condiments and preservatives, packed as above and analyzed at periodic intervals for sensory and bacteriological characteristics during storage.

RESULTS AND DISCUSSION

Table 1 gives the composition of the modified preservative mixture. Reduction of salt to 2% decreased the shelf life when calcium propionate was the only preservative. Hence sorbic acid was incorporated. Citric acid, which was used in the earlier mixture was substituted with tartaric acid as it is the acid present in tamarind fruit, one of the traditional ingredients in fish preparations.

Table 2 gives the changes in organoleptic quality of six species of fish during storage at ambient temperature. These results show that during the first 24 hours the quality of the fish is the same as it was initially, after 48 hours quality is fair, and after 72 hours it is poor to unacceptable.

Table 3 shows the progressive spoilage of treated black kingfish slices. The total bacterial count decreases to hundreds 24 hours after the treatment and then gradually increases until after the third day it reaches the initial levels. The levels of TMA and TVN are also within limits. Thus from the results in Tables 2 and 3 it can be seen that treated fish slices kept well for 1-3 days. All the consumers are unanimous in their opinion that the treated fish slices were in excellent condition after one day and were acceptable up to three days.

Table 4 gives the moisture contents of the fried mussels and their shelf life both for control and treated samples. These data show that at higher moisture levels, treated products have a better shelf life than the controls whereas at lower moisture levels the effect is not so pronounced. Table 5 shows the composition of the preservative mixture used for fried mussels. Tartaric acid was excluded from the mixture as it imparted an acidic taste to the product. Pepper and coriander powders were added to suit the local taste. Table 6 presents the proximate composition of the fried product and Table 7 its quality changes during storage at ambient temperature.

Calcium propionate at 3000 ppm is added in the flour for bread making in many countries (Ranken, 1988). Hence, its presence at the same level in fish may not be harmful. Sorbic acid at 0.2% is added in various food products to prevent fungal attack and to prevent proliferation of some pathogenic bacteria. Tartaric acid is known to be harmless. Hence, the preservative mixture can be assumed to be of no health hazard to the consumer.

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Table 1 Composition of the modified preservative mixture

Ingredients	Percentage by weight of fish to be treated
Calcium propionate	0.3
Sorbic acid	0.2
Tartaric acid	0.2
Refined common salt	2.0
Chilly powder	3.0
Turmeric powder	0.2

Table 2 Changes in organoleptic quality of six species of treated fish during storage at room temperature

Species of fish	Storage time in days			
	0	1	2	3
Mackerel tuna (*Euthynnus affinis*)	Excellent	Excellent	Good - fair	Poor - off taste
Black kingfish (*Rachycentron canadus*)	Excellent	Excellent	Good	Good - fair
Drab jew fish (*Johnius sina*)	Excellent	Excellent	Good - fair	Poor - off taste
Indian Mackerel (*Rastrelliger kanagurta*)	Excellent	Excellent	Good - fair	Off taste
Indian Oil Sardine (*Sardinella longiceps*)	Excellent	Excellent	Good - fair	Off taste
Seer fish (*Cybium commersoni*)	Excellent	Excellent	Good	Good - fair

Table 3 Changes in quality of treated Black Kingfish portions during storage at room temperature

Quality characteristics	Storage time in days				
	Before treatment	After treatment	1	2	3
SPC/g	4.857×10^3	9.664×10^3	3.25×10^2	2.062×10^3	1×10^6
Faecal streptococci/g.	272	396	52	58	315
Coliforms/g.	190	33	38	4	194
TVN (mg%)	-	11.2	14.0	25.2	42.0
TMA (mg%)	-	2.8	2.8	5.6	7.0
Organoleptic quality	-	Excellent	Excellent	Good - fair	fair

Table 4 Effect of moisture of fried mussels on shelf life

Moisture %	Shelf life (days)	
	Control	Treated
40.0	4	8
30.0	5	12
15.0	45	>60

Table 5 Composition of the preservative mixture for fried mussel meat

Ingredients	Percentage by weight of fried mussels
Refined common salt	2.0
Calla powder	3.0
Tumeric powder	0.2
Pepper powder	0.2
Coriander powder	0.75
Calcium propionate	0.3
Sorbic acid	0.2

Table 6 Proximate composition of fresh mussels and fried mussel meat

Parameters	Fresh	Fried
Moisture %	85.0	12.50
Protein %	8.40	52.50
Fat %	1.20	18.00
Ash %	1.40	7.40

Table 7 Changes in quality of fried mussel meat during storage at ambient temperature

Storage period in months	SPC/g	Sensory characteristics	Moisture %
0	Less than 100	Crisp, very good	9.20
1	"	Crisp, very good	9.60
2	"	Crisp, very good	10.85
3	"	Crisp, very good	10.65
4	"	Less crisp, slight old taste	-
5	"	Less crisp, acceptable	11.85
6	"	Less crisp, off flavour	12.46

FORMATION OF HISTAMINE IN INDIAN MACKEREL
(*Rastrelliger kanagurta*)

by

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ABSTRACT

Histamine formation in Indian mackerel at three temperatures of storage 2 ± 1 °C in flake ice, 10 ± 1 °C and ambient temperature 26 ± 1 °C was studied. The formation of histamine was not significant up to a period of 10 hours, at ambient temperature, reaching only an average value of 7.51 mg/100g. fish and increases significantly thereafter. In iced fish for a period up to 72 hours the production of histamine was not significant whereas fairly substantial amounts were produced after 56 hours at 10 °C. A correlation between the content of histamine and the overall quality of fish was not seen, the fish becoming unacceptable even when the histamine levels were very low.

INTRODUCTION

Fishes of the scombroid group are known to cause histamine poisoning and because of this fact scombroid poisoning has been synonymously used to represent histamine poisoning. Histamine poisoning causes health hazards like urticaria, localised inflammation, diarrhoea, vomiting, palpation, hypotension, burning and itching, nausea, headache, etc. Fish in general, being a source of histamine poisoning, several countries have pronounced allowable safety levels of histamine in fish and fish products. Many countries, especially European countries, have accepted a histamine level of 10 to 20 mg/100g fish as the maximum allowable level and 50 mg/100g fish as the hazard action level (Anon, 1987).

Studies on histamine formation show that the production of histamine depends on the free histidine content of the fish and the histidine decarboylase activity of the bacteria (Arnold and Brown, 1978). Edmunds and Eitenmiller, 1975; Niven *et al*, 1981). Several studies have been made on production of histamine along with putrescine and cadaverine at different storage temperatures. It has been observed that histamine producing bacteria were killed or unable to produce histamine at low temperatures, 0-10 °C (Ota and Kaneko, 1958; Edmunds and Eitenmiller, 1975). Some workers have reported that small amounts of histamine were formed when the fish was stored at 2-6 °C depending on the humidity of the environment and that no histamine was formed at -20 °C even in two months time (Dabrowski, 1968; Cattaneo *et al*. 1978; Baldrati *et al*, 1980). All studies seem to agree that significant levels of histamine leading to histamine toxicity are formed at temperatures above 15 °C (Kimata and Kawai, 1953; Edmunds and Eitenmiller, 1975; Hardy and Smith, 1976; Park *et al*, 1980; Smith *et al*, 1980; Baldrati, 1980).

In India fish is landed by country craft without much preservation. Studies with ocean fresh fish are really needed. Further, not much work has been done on histamine in Indian fishes. Kalyani and Bai (1965) studied histamine formation during spoilage and storage in some teleosts. The incidence of histidine decarboxylase bacteria in fish and fish environs and the level of histamine in two scombroid fishes, *Scombermorus guttatus, Rastrelliger kanagurta* has been reported by Subburaj *et al* (1984). Ames *et al* (1987) studied histamine formation in Indian oil sardine at ice storage temperature, 10 °C, 23 °C and 31 °C. Apart from this, practically no work has been reported from India. This work is an attempt to observe the histamine formation at three temperatures of storage such as ice storage temperature (2°C), 10°C and ambient temperatures (26°C).

MATERIALS AND METHODS

A part of the work, comprising collection and storage, was done on board the research vessel "FORV Sagar Sampada". "FORV Sagar Sampada" is a 71.5m Fisheries and Oceanographic Research Vessel owned by the Department of Ocean Development, Government of India. The vessel is operated for fisheries resources survey, marine meteorological and fish utilization studies. Fresh mackerel caught in one of the trawl net operations off the Kakinada coast was utilised for the study. Storage and sampling was carried out in the processing laboratory in the ship. Immediately on collection the fish was thoroughly washed with fresh water and divided into three lots as explained below:

A. Mixed with an equal quantity of flake ice and stored in an ice box and the temperature was maintained at 2 ± 1°C.
B. Kept stored in a refrigerator maintained at 10 ± 1°C.
C. Kept stored in an open tray and left on the deck of the ship under shade, at ambient temperature, 26 ± 1°C.

Samples were drawn every 8 hour interval and was continued for 72 hours in the case of fish stored at 2°C and 10°C. In the case of fish stored at ambient temperature, sampling was carried out every 2 hours for 12 hours and after every 4 hours for the remaining 20 hours by which time the fish became putrid and foul smelling. Apparent quality of the fish was recorded and kept in the freezer storage of the ship maintained at -20°C. Histamine assay was carried out by the method of Hardy and Smith (1976).

RESULTS AND DISCUSSION

The quality of fish stored at ice storage temperature (2°C), 10°C and ambient temperatures (26°C) and histamine formed under these conditions are presented in Table 1.

At ice storage temperature (2°C) no significant quantity of histamine was formed for the entire period of storage (76 hours) (Table 1). Small quantities of histamine were formed at 10°C and as the storage period increased, significant amounts of histamine were formed (histamine level above 20 mg/100g. fish). Fish samples stored for 64 hours at 10°C developed 64.71 mg. histamine per 100g. fish. A decrease in histamine level was observed in samples stored beyond 64 hours (13.41 mg/100g fish). At ambient temperature (26°C) the histamine formation increased rapidly after an induction period of 10 hours. The level of histamine increased to 274 mg/100g. fish during 28 hours storage at 26°C, while it was only 7.51 mg/100g fish at 10 hours (Table 1).

Induction period

Figure 1 shows the average values of histamine at different storage temperatures against time. An induction period during which no histamine or very little histamine is produced was observed in this study. Such an induction period has been observed for histamine formation in other fish (Arnold and Brown, 1978; Ames *et al*, 1987). At 10°C the induction period was extended to 56 hours and a rise in histamine production was observed at 64 hours (21.15 mg/100g. fish). At 16°C, the induction period was reduced to 10 hours. The induction period may be considered as the minimum time required for the accumulation of a sufficient quantity of enzyme.

Ames *et al*, (1987) observed that no significant amounts of histamine were formed in Indian oil sardine at ice storage temperature of 11 days, with histamine averaging 1.0 mg/100g. fish. At 10°C, the above authors observed no significant amounts of histamine for 6 days, with an average of 1.5 mg%. In the present study on Indian mackerel no significant amounts of histamine were found at ice storage temperature for 72 hours. But appreciable levels of histamine may be formed in Indian mackerel at 10°C after an induction period of 56 hours. This is in agreement with the observations of Ota and Kaneko (1958), Edmunds and Eitenmiller (1975), Kalyani and Bai (1965). The above authors concluded their work with the observation that histamine forming bacteria were killed or unable to produce histamine at low temperatures ranging from 0 to 5°C and that small amounts of histamine were formed at temperatures of 6 to 10°C.

Large amounts of histamine were formed at high storage temperatures (Kimata and Kawai, 1953; Baldrati *et al*, 1980; Frank *et al*, 1981). Kimata and Kawai observed 826 mg. histamine per 100g. fish in Pacific mackerel stored for 46 hours at 23°C and in a similar study by the same authors (1953b), 354 mg histamine per 100g. fish was observed in mackerel fillets stored for 75 hours at 17°C. Baldrati *et al* (1980) observed a histamine level of 67 mg/100g. fish in mackerel stored for 3 days at 18°C. This investigation indicates that temperatures in the range 17-26°C are specific for histamine production in fish. Salguero and Mackie (1979) suggest that at higher storage temperature conditions exist for the growth of bacteria capable of producing relatively large amounts of histamine. The above observations reinforces the present finding of very high levels of histamine (174 mg%) in Indian mackerel stored for 28 hours at 26°C. It is also important to note that relatively high amounts of histamine are formed within a short period of 20 hours at ambient temperature (26°C).

The results show that fresh mackerel do not contain histamine and when stored under sufficient ice do not develop significant levels of histamine for an appreciable length of time. High amounts of histamine were formed at 10°C when kept for periods more than 56 hours. Very high levels of histamine are formed only after an induction period of 10 hours at ambient temperature (26°C).

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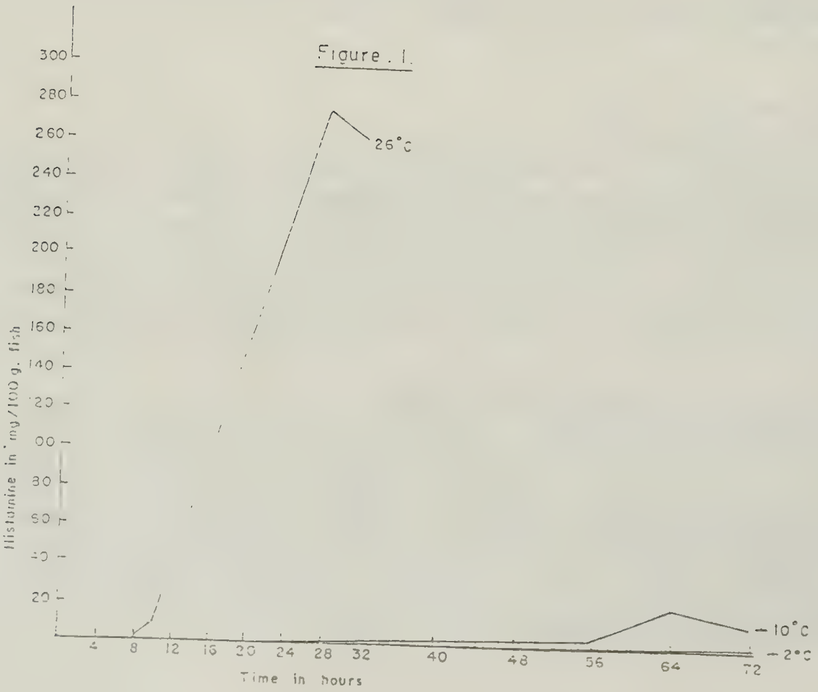

Figure. 1.

Table 1 Histamine in mackerel stored under different storage conditions

Storage condition	Time hrs.	Quality	No. of samples	Histamine mg/100g. fish range	Mean
Ice storage temp. 2°C	8	Excellent	4	0-0.44	0.11
	16	Excellent	4	0.1-0.86	0.45
	24	Good	4	0.57-1.62	0.60
	32	Good	4	0-0.84	0.54
	40	Good	4	0-4.28	1.46
	48	Good	4	0.29-1.44	0.83
	56	Fair	4	0-0.78	0.20
	64	Fair	4	0-0.88	0.58
	72	Fair	4	0-0.74	0.41
10°C	8	Excellent	4	0-0.34	0.20
	16	Good	4	0-1.29	0.65
	24	Good	4	0.68-1.24	0.94
	32	Gair	4	1.28-2.09	1.71
	40	Fair	4	1.66-3.01	2.14
	48	Fair	4	2.82-4.60	3.40
	56	Poor	4	4.22-8.72	6.15
	64	Poor	4	4.62-64.71	21.15
	72	Poor	4	4.48-29.25	13.41
26°C Ambient temp.	4	Excellent	2	0-0	0
	6	Good	2	0.18-0.20	0.10
	8	Fair	2	0.48-0.67	0.58
	10	Fair	4	0.77-11.31	7.51
	12	Poor	4	5.59-42.74	30.00
	16	Bad	4	86.14-103.32	92.25
	20	Bad	4	151.15-194.82	180.00
	24	Bad	4	216.41-261.47	234.00
	28	Bad	4	267.20-287.36	274.00
	32	Bad	4	242.84-272.18	260.00

THE MICROBIAL FLORA DEVELOPING ON INDIAN MACKEREL (*Rastrelliger kanagurta*) DURING THAWING AT DIFFERENT TEMPERATURES

by

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ABSTRACT

The changes in the number and types of bacteria on Indian mackerel (*Rastrelliger kanagurta*) were evaluated at three thawing temperatures namely 29 ± 2°C (room temperature), +15°C and +4°C. The multiplication rate of the bacteria increased with increase in external temperature. The diverse nature of the flora seen in the beginning, gave way to the accumulation of a single type in the end. The isolates were identified as *Pseudomonas* spp. and the biochemical activity and spoilage potential confirmed. No correlation was observed between organoleptic score and total plate count. The studies confirmed the existence of lag in the growth rate of bacteria in frozen fish compared to fresh fish. The changes in the bacterial flora of frozen fish during the process of thawing indicates a close similarity to the changes in microbial flora of fish during chill storage.

INTRODUCTION

Frozen sea foods are accepted as highly stable and less prone to attach by bacteria. However, when frozen fish is defrosted, surviving bacteria are liberated and begin multiplication as in the normal growth cycle.

Spoilage of thawed foods depends on many intrinsic as well as extrinsic factors such as condition of food material environments etc. (Hayes, 1985). Sulbacher (1952) noticed that during thawing frozen vegetables spoiled more rapidly than fresh ones. On the other hand, he noticed no difference in the spoilage rate of the fresh and frozen meat. The spoilage pattern of frozen thawed fish is reported to be much similar to the fresh ones (Shewan, 1956, Liston, 1980).

Bacteriological changes undergone during the process of thawing was studied in fish and other products (Pivnick, 1949; Sulbacher, 1952; Hayward and MacCallum 1969; Howard *et al*, 1970).

This study presents the major changes taking place in the microflora during the process of thawing of mackerel (*Rastrelliger kanagurta*) and brings out the possible relation of these changes to the nature and activities of bacterial isolates.

MATERIALS AND METHODS

The sample consisted of mackerel having a body weight above 85k (large) collected from the fishing craft of Cochin, in rigour under ice and having organoleptic quality according to IS: 6032, 1971. The material, after collection was reiced with an ice to fish ratio of 1:1 and brought to the laboratory. Potable water containing 5 ppm available chlorine was used to clean the fish.

Block freezing was used in this study. 6-8 fish were soaked in duplex cartons lined inside with 100 gauge polythene paper. Sufficient chilled water was used to cover the fish. The material was frozen in contact plate freezer (API, Jackstone) at -40°C, glazed with chilled potable water and stored at -20 ± 2°C in deep freezer.

Samples were taken out when required. Thawing was accomplished at three temperatures viz. 29 ± 2°C (room temperature-RT) +15°C and +4°C. The sample was placed inside polythene bag and kept in running water (for RT) and cabinets of refrigerators maintained at desired temperature.

For bacteriological analysis, the method outlined in FDA (1978) was followed. Tryptone glucose extract agar (Difco) was used as plating medium. Colony counts were taken at the end of 48 h. at 29 ± 2°C (RT). Along with the skin and muscle of fish, the drip was also analyzed.

Colonies were isolated from plates and identified up to generic level as per the schemes (Shewan *et al*, 1960; Buchanan and Gibbons 1974; Lee and Pfiefer, 1975).

The spoilage capacity of the isolates were determined by studying their activity in fish press juice (Lorke *et al*, 1975) and also by biochemical tests (Harrigan and McCance, 1978).

For organoleptic scoring, a 10 point product quality scale was used for measuring the acceptability of the product. The scoring was 10 excellent, 9 very good, 7 good, 5 medium, 3 poor, 1 very poor and 0 extremely poor.

Along with the frozen fish fresh uniced fish was also placed at the three test temperatures and evaluated as in the case of frozen fish.

RESULTS AND DISCUSSION

Frozen mackerel, stored at -20 ± 2°C for four months were used in this study. The changes in the number of bacteria per gram of the skin with muscle portion of the fish and per ml. of the drip are presented in Table 1.

An examination of the data reveals that the multiplication rate of bacteria is lowest at 4°C in the frozen sample. The control sample also behaved in more or leas similar manner. The total plate count registered peak values at room temperature in both thawed fish and control.

Compared to frozen fish the multiplication rate of bacteria was faster in the control sample. The mean generation time of the total microbial population in frozen fish, calculated on the basis of total plate count in Table 1 was 1.8, 3.5 and 61.6 after 24 h at RT, 15°C and 4°C. The corresponding values for the control sample were 1.53 and 2.6 at RT and 15°C respectively. For control samples kept at 4°C, no growth was noticed within 24 h.

These values suggest the possibility of existence of an extended lag period in the growth cycle of bacteria from frozen fish. In this case the lag period was found to be 9 to 16 h. at RT and 15°C. A lag period of 2-3 days was observed for macaroni and cheese at 5°C by Peterson (1962). Munce and Buckle (1981) noted a lag period of one day for macaroni and peas. According to Lawton and Nelson (1954) this lag period is not characteristic of the microorganism present, but rather the existing physiological condition of the product.

The drip which exuded from the fish registered an increase in TPC with increase in keeping time. The drip showed a greater multiplication rate of bacteria especially toward the end of the period. The ready availability of nutrients in the drip may be the reason for the enhanced growth rate.

Table 2 gives the organoleptic scoring of the thawed fish and the control on the basis of this, the limit of acceptability at RT is between 9 and 16 h. If the bacterial count of 10^7 CFU/g is taken as the limit of acceptability for tropical fish (Setty *et al*, 1985) the thawed fish is acceptable up to 24 h. at room temperature. Hence there exists poor correlation between bacterial count and organoleptic tests in the case of frozen-thawed fish.

The changes in the bacterial groups during the process of thawing are presented in Table 3.

On the onset of the thawing process, the bacterial genera were very selective, comprising the survivors of the freezing process. The gram positives, mainly *Micrococcus* spp. constituted the bulk of the flora.

With the progress of the thawing time, there was shift in the bacterial genera from the gram positive to the gram negative types. At the end of 24 hours when the fish was completely spoiled. *Pseudomonas* spp. constituted the major fraction in the muscle as well as the drip. At the other temperatures of thawing the predominant bacterial type was *Pseudomonas* spp. indicating that thawing temperature has no selective influence on this bacteria.

The *Pseudomonas* spp. have been considered as active spoilers of wet fish (Castell *et al.* 1957; Shaw and Shewan, 1968; Miller *et al*, 1972). In the case of chill storage of marine tropical fish (mackerel) the terminal flora at the time of spoilage is *Pseudomonas* spp. (Surendran *et al*, 1989). Recent studies on microbial associations have confirmed that in foods having intrinsic properties, optimum growth for a wide range of microorganism and having a buffering system which prevents extreme conditions, the spoilage organisms tend to be Pseudomonas . They are also very much adapted to degradative changes of carbon and nitrogen cycles (Board, 1983).

The biochemical characteristics of the *Pseudomonas* isolates from this study showed them to be very active (Table 4). The behaviour of these isolates in fish press juice after incubation for 4 and 10 days confirmed the spoilage potential of the isolates (Table 5).

The incubation temperature and the period has a striking influence on the biochemical tests. As evident from the results, the biochemical activity of the isolates was very slow at 4°C. This may be due to the slower growth rate at this temperature compared to other temperatures. A relation between spoilage and cell count has been noted by Liston (1964). According to Ingram (1971) the amount of chemical change produced by a single cell is so small that alterations detectable by chemical means can be produced only by a cell populations reaching maximum numbers.

This study also supports the statement (ICMSF, 1980 and Hayes, 1985) that there is some relationship between the initial flora of the raw material before freezing and flora at the time of spoilage of the thawed product. The *Pseudomonas* spp which was the predominant bacterial genera in thawed mackerel at the time of spoilage constituted good portion of the microbial flora of the raw mackerel also (Thampuram 1988).

These results bring forth the existence of a close similarity between frozen thawed mackerel and chilled mackerel especially on the build up of the bacterial genera.

Table 1 Effect of different thawing temperatures and thawing periods on the total plate count/g of mackerel (*Rastrelliger kanagurta*)

Thawing period h.	Thawing temperature °C	TPC of the frozen fish		TPC of the control skin with muscle
		Skin with muscle	Drip	
0		5.18x10^{3}	Nil	2.11x10^{4}
	4	5.6x10^{3}	Nil	1.0x10^{4}
2	15	9.4x10^{4}	Nil	9.8x10^{3}
	RT	1.1x10^{4}	Nil	6.4x10^{4}
	4	5.4x10^{3}	Nil	19.8x10^{3}
9	15	1.2x10^{4}	Nil	1.2x10^{4}
	RT	7.2x10^{4}	2.9x10^{3}	1.8x10^{6}
	4	4.8x10^{3}	Nil	8.6x10^{3}
16	15	7.8x10^{4}	1.8x10^{2}	3.8x10^{5}
	RT	3.7x10^{6}	7.8x10^{6}	4.2x10^{8}
	4	6.8x10^{3}	Nil	1.4x10^{4}
24	15	5.71x10^{5}	2.8x10^{5}	1.1xl0^{7}
	RT	5.21x10^{7}	8.7x10^{9}	1.1x10^{9}

Table 2 Organoleptic scoring of frozen-thawed mackerel and the control

Thawing time hrs.	Frozen mackerel thawed at °C			Unfrozen sample kept at °C		
	4	15	29 ± 2 (RT)	4	15 (RT)	29 ± 2
0	8	8	8	9	9	9
9	8	8	7	9	8	7
16	8	7	4	9	7	4
24	8	6	3	8	6	3

Table 3

Thawing temp. °C	Thawing period hrs.	Bacterial species as %													
		Pseudomonas		Moraxella		Acinetobacter		Flavobacterium		Micrococcus		Others		Total isolates	
		M*	D*	M	D	M	D	M	D	M	D	M	D	M	D
Room temp. 29 ± 2°C	0	14	0	12	0	14	0	7	0	51	0	2	0	58	0
	9	30	60	16	2	16	2	12	0	24	3	2	0	60	68
	16	62	72	15	7	3	1	3	0		6	0	0	62	58
	24	72	80	10	4	11	0	2	0	17	2	0	0	61	55
											0				
										51	6				
15°C	0	17	0	16	0	13	0	5	0	49	0	0	0	71	0
	9	36	0	15	0	11	0	10	0	28	0	0	0	75	0
	16	60	76	17	1	3	0	4	0	16	2	0	0	58	48
	24	76	82	8	3	9	5	5	0		3	0	0	60	48
										2	1				
											0				
4°C	0	20	0	16	0	11	0	6	0	47	0	0	0	63	0
	9	40	0	18	0	10	0	11	0	21	0	0	0	68	0
	16	67	0	14	0	3	0	2	0	14	0	0	0	53	0
	24	78	0	10	0	8	0	4	0	0	0	0	0	57	0

*M = Muscle with skin *D = Drip

Table 4 Biochemical characteristics of the *Pseudomonas* isolates

Biochemical tests	Percentage of isolates showing positive reaction at temperatures °C		
	4	15	29 + 2 (RT)
Production of hydrogen sulphide	64	65	69
Production of indole	4	5	5
Liquefaction of gelation	52	51	52
Hydrolysis of casein	21	24	29
Phosphatase	40	43	43
D-Nase	17	19	19
Reduction of trimethylamine oxide	38	44	32
Utilization of: glucose			
aerobic	71	71	71
anaerobic	0	0	0
Production of acid from:			
glucose	93	94	94
sucrose	93	94	94
lactose	0	0	0
maltose	48	56	56
mannitol	6	8	10
Total isolates	126	126	126

Table 5 Activity of the *Pseudomonas* isolates in the fish press juice

Biochemical tests	Incubation period days	Percentage of isolates showing positive reaction at °C		
		4	15	29±2 (RT)
Production of ammonia	4	0	6	25
	10	52	77	77
Production of hydrogen sulphide	4	18	54	54
	10	65	73	73
Production of trimethylamine	4	17	17	18
	10	40	49	53
pH rise	4	8	17	38
	10	79	100	100
odour	4	13	16	22
	10	79	86	87
TPC/ml	4	10^4	10^5	10^6
	10	10^6	10^9	10^9
Total isolates		126	126	126

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THERMAL EFFICIENCIES OF CONTAINERS FOR TRANSPORTATION OF ICED FISH

by

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ABSTRACT

In India iced fresh fish is usually transported from landing centres to urban markets in bamboo baskets lined inside with sal (*Shorea robusta*)leaves and overwrapped with gunny or in second hand plywood or wooden boxes of capacities 40 to 120 kgs. There is no fixed ratio of ice to fish and replenishment of ice is done en route in some cases where the transportation exceeds 24 hours. The mode of transport is by uninsulated rail wagons or insulated/tarpaulin covered trucks by road. Normally no pre-chilling of fish is done prior to packing. Due to high ambient temperatures and the poor insulating qualities of commercially used containers, the temperatures of fish on arrival at destinations is as high as 18°C.

INTRODUCTION

CIFT has for the past several years studied the commercial practices of packaging of fresh fish and has developed insulated containers or modified the existing containers, with a view of reducing the rate of melting of ice and thus extend the shelf life of fresh fish. This paper deals with the studies on thermal efficiencies in terms of ice melting rate index of commercial and modified containers developed, in the Institute, and recommendations of suitable containers for a specific transit time. The thermal efficiency of the container was determined based on the simple methods of Boeri (1985) and Lupin (1985), by studying the rate of melting of ice in the container completely filled with ice under steady ambient conditions.

The quantity of heat transferred from the exterior into the container, filled completely with ice, under steady ambient conditions is given as (temperature inside assumed as 0°C):

$Q = AU\,{}^{T}e$ Kcal/hour
where A is external surface area m^2
U is overall heat transfer coefficient K cal/hour/m^2/°C
${}^{T}e$ is external temperature °C

This heat is counterbalanced by the heat resulting from the melting of ice

$$Q = \lambda \frac{d^{M}i}{dt}$$

where λ is the latent heat of fusion of ice in kcal/kg

${}^{M}i$ = quality of ice kg

t = time (hours)

Equating these two equations

we have $M_i = M^\circ_i - \left[\frac{AU}{\lambda} T_e\right] t$

where $M^\circ_i - K^t$

where K is the expression in brackets which is the melting rate constant of the given container

or $U = \frac{M^\circ_i - M_i \ \lambda}{A T_e t}$ which is the effective insulation efficiency of the container.

The containers packed with ice were kept under ambient conditions and ice melted was measured every two hours during which period the temperature fluctuations were within ±1°C. For the shelf life studies of iced fish, the fish was chilled in ice for 2 to 4 hours depending on size of the fish until it attained 0-1°C prior to packing. Subsequently fish and ice were packed in 1:1 ratio, in alternate layers. About 30-40% of the ice required was kept as a top layer where there is a chance of maximum heat leakage and the rest evenly distributed in different layers. The temperatures of fish in the different layers and ambient temperatures were recorded continuously with a multichannel temperature recorder and thermocouples. At the end of the experiment the quality of fish was judged by organoleptic and sensory evaluations and the experiment was stopped when fish attained a maximum temperature of 5°C. The table gives the overall thermal efficiencies of different containers in terms of ice melting index and the shelf lives of pre-chilled iced fish (1:1).

It is recommended that under tropical conditions, the ratio of fish to ice has to be maintained 1:1 and for maximum utilization of the container volume, the fish has to be chilled for 2-4 hours until it attains 0-1°C prior to packing. Fish and ice are to be packed in alternate layers, (fish layer not exceeding 10 cms) with 10% more ice on the top layer.

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Table 1

Sl. No.	Description of container	Heat transfer coefficient Kcal/hr/°C/m^2	Ice melt index kg/hr/°C	Storage life of fish in marketable condition (hr)*
1.	Bamboo basket lined inside with Sal leaves and gunny overwrap (60 litres capacity)	2.312	0.0185	24
2.	Bamboo basket lined with 250g polythene either side and gunny overwrap (60 litres capacity)	1.907	0.01525	30
3.	Bamboo basket with 20 mm foamed polyethylene inside and gunny overwrap (60 litres capacity	1.17	0.01	42
4.	Corrugated polypropylene box with 25 mm foamed polyurethane (18 litres capacity)	0.63	0.00367	40
5.	Galvanised iron box with 50mm foamed polystyrene (100 litres capacity)	0.519	0.014	56
6.	3 ply corrugated fibre board box, polythene coated either side (38 litres capacity)	2.02	0.02	13
7.	Plywood box with 2.5 cm foamed polystyrene insulation (46 litres capacity)	0.547	0.0075	42
8.	G.I. box with 2.5 cm foamed polystyrene (65 litres capacity)	1.6	0.025	19

* At ambient temperature 26-34°C maximum temperature attained by fish at the end of storage period 5°C.

USE OF FOOD PRESERVATIVES IN SALTED DRIED FISH

by

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ABSTRACT

The reduction of microbial deterioration in salted dried fish by application of the food preservatives, sodium sorbate, sodium benzoate, sodium, propionate and sodium bisulphite was assessed. *Halobacterium sp* was isolated from Indonesian salted dried fish as a test organism. The bacterium was grown on complex halophilic medium (CHM) and salted dried fish homogenate (SDFH). Effectiveness of food preservatives was also tested on salted dried fish samples.

Halobacterium grown on CHM and SDFH was not sensitive to sodium bisulphite at the highest levels tested 0.3%, but sodium benzoate at a level of 0.10% was lethal to the bacterium grown in those two media. The lag phase was extended for 7 days, in CHM, but no growth was observed in SDFH. Salted dried fish previously dipped in a 1% solution of either sodium benzoate or sodium sorbate showed no sign of spoilage by *Halobacterium* after 17 days incubation.

INTRODUCTION

Curing by salting and drying or a combination of these techniques is employed in South East Asia countries including Indonesia. Such a processing technique is not only cheap and relatively easy to practice, but also yields a product with a relatively long shelf life. Fish curing is often the only method available to processors in rural areas.

There are some problems associated with traditionally processed fish products, mainly resulting from the growth of halotolerant and halophilic bacteria. The halophile grows only on the surface of salted dried fish producing a pink to red colony resulting in an undesirable appearance and strong smell. Bacteria including *Micrococcus spp, Staphylococcus spp.* and *Halobacterium spp.* are reported as the major microflora (Sanderson *et al.* 1987).

It is evident that consumption of salted dried fish may increase the level of salt intake leading to cardiovascular problems. The application of preservatives during processing might reduce the necessity for high salt concentration in extending shelf life resulting in healthier products.

MATERIALS AND METHODS

Materials

Halophilic bacteria: Bacteria from salted dried fish (jambal roti) were isolated. It was identified as *Halobacterium sp.* which was maintained on 22.5% salt Complex Halophilic Agar (CHA). This bacterium was incubated at 37°C.

Media: The media used were CHM designed by Eimhjellen (1965) with a little modification and salted dried fish homogenate (SDFH).

Fish: Cod (*Gadus morhua*) was used to make salted dried fish homogenate as well as salted dried fish.

Food preservatives: The four food preservatives evaluated were sodium benzoate, sodium propionate, sodium bisulphite and sodium sorbate.

Methods

Experimental design and analysis of data: Procedures were based on a split-plot design (SPD) which employed two factors - food preservatives and storage time. Data collected were analyzed by analysis of variance (Anova) and the least significant different (LSD).

Growth of *Halobacterium sp* in CHB and SDFH containing food preservatives : The experiment for each food preservative took approximately eight days and employed three concentrations. The preservatives were tested with this bacterium. The concentrations used for both sodium sorbate and sodium benzoate were 0.1%, 0.15% and 0.2%; for sodium propionate 0.1, 0.2 and 0.3%; and sodium bisulphite 0.01, 0.02 and 0.03%. Both media containing food preservatives were inoculated with 10^3/cells of the bacterium. The media were incubated at 37°C and withdrawn at 24 hour intervals. Enumeration was done by spread plate technique and a colorimeter, using a wavelength of 540 nm (growth of bacterium in CHM only). To prepare SDFH, 100 g of salted dried fish homogenate powder and 22.5% salt solution were made up to one litre. The remaining procedures were the same as those described in the preparation of growth in CHB. SDFH was used as the growth medium, instead of CHM.

Growth of *Halobacterium sp* on salted dried fish (SDF): The concentrations of NA-sorbate and Na-benzoate evaluated were 1%, 2% and 3% and Na-bisulphite was tested at concentrations of 0.2%, 0.4% and 0.6%. SDF was sterilized, dipped into the food preservatives followed by dipping in 25% salt solution containing 10^4/cells/ml of *Halobacterium.* The samples were incubated at 37°C and withdrawn for TPC analysis at 48 hour intervals.

RESULTS AND DISCUSSION

Growth of *Halobacterium* sp in CHB supplemented with sodium bisulphite

Results of growth of *Halobacterium* in CHB are presented in Figure 1. None of the treatments delayed initiation of growth, after 3 days of incubation. Reddening occurred on day 4, when growth achieved the maximum number. On the next day, growth began to decrease and the broth produced a very strong off-odour. So, Na bisulphite at the highest concentration tested did not significantly inhibit growth of this bacterium. Lueck (1980) reported that most bacteria are sensitive to Na bisulphite at levels of 50-100 ppm (0.005% to 0.01%). *Acetobacter* and *Lactobacillus* (Amerine, 1980 and Jay, 1986) were killed by this preservative at a level of 100 ppm. *Salmonella* is inhibited at levels of 15-109 ppm (Lueck, 1980).

Growth of *Halobacterium sp* in CHB and SDFH supplemented with Na-propionate

Result of growth of this bacterium in CHB & SDFH are shown in Figures 2 and 3.

Like Na bisulphite, Na propionate was not effective against Halobacterium grown in CHB at the highest level tested, 0.3%. The viable counts of the three concentrations tested were not significantly lower than those of the control when this bacterium was grown in CHB. However, the preservative significantly inhibited growth when the bacterium was grown in SDFH. Its increased effectiveness may be due to reduced levels of nutrients in SDFH. Although use of Na-propionate at the maximum level tested (0.2%) was able to inhibit growth, this was not sufficient to delay the reddening in the homogenate or off-odour which occurred on the same day as the control (day 5). The onset of reddening occurred one day later than in CHB.

Growth of *Halobacterium sp* in both CHB and SDFH supplemented with Na-sorbate

Results of the effect of Na-sorbate on *Halobacterium* grown in both CHB and SDFH are presented in Figures 4 and 5 respectively. Na-sorbate proved very effective against this bacterium. At all levels tested, growth of the bacterium in CHB was persistently inhibited. Viable count for the lowest concentration (0.1%) was significantly lower than the control, throughout the incubation period. However, this preservative did not completely stop growth within the range of concentrations tested.

Growth in SDFH was significantly inhibited at all levels of concentration tested in the first 24 hours, and bactericidal effect resulted in decreasing viable counts. After 1 day incubation, the numbers increased again, but they were still below the initial population (4 log/ml) until the end of incubation (6 days). Reddening did not develop during the incubation period. At the highest level tested (0.2), this preservative was not lethal to the bacterium. Robach (1978) reported that at this level it was lethal to *Pseudomonas fluorescence*, a common agent of fish spoilage.

Growth of *Halobacterium sp* in CHB and SDFH supplemented with Na-benzoate.

Results of the effect of Na-benzoate on growth of *Halobacterium sp* are presented in Figure 6. It is clear that Na-benzoate considerably inhibited growth of this bacterium and was even bactericidal. The numbers decreased and no growth was observed between 2 and 5 days of incubation, but the bacterium appeared again on day 6 for 0.10%, day 7 for 0.15% and 0.20% Na-benzoate. Reddening did not occur until the end of incubation.

Results of the effect of Na-benzoate on the bacterium grown in SDFH are presented in Figure 7. Viable counts for all treatments decreased with increasing concentration of sodium benzoate. No growth was detectable, starting on day 2 for 0.1%, and day 3 for 0.15% and 0.2%. These results confirm that sodium benzoate did not only inhibit growth, but also destroyed the bacterium. Consequently, reddening never occurred in SDFH at all levels of sodium benzoate tested.

Use of food preservatives in fish processing

Previous results indicated that the order of effectiveness of the anti-microbials tested was Na-benzoate, Na-sorbate and Na-propionate. Na-bisulphite at permitted levels had no effect on *Halobacterium*. However, it was retested because of its importance as a food preservative in the fish curing industry. Results of the use of Na-bisulphite in salted and dried fish are shown in Figure 8.

When the three groups of salted dried fish were dipped into 0.2%, 0.4% and 0.6% of sodium-bisulphite solutions, residues for the three treatments approximated 0.012%, 0.023% and 0.035% respectively. In general, treatments moderately inhibited growth of *Halobacterium*, throughout the incubation period, leading to lower viable counts than the control. As previously discussed, at the maximum level of 0.03%, sodium bisulphite did not effect growth when the bacterium was grown in CHB and SDFH. This may be explained because although total residue levels in fish, for example the 0.2% treatment, was only 0.012% the concentration would be higher on the surface of SDF where this bacterium normally grows. This suggests that this bacterium might after all be susceptible to sodium bisulphite at high concentration. Reddening for 0.25%, 0.4% and 0.6% treatments occurred in the same day which was one day earlier for the control (day 10). This suggests that sodium bisulphite should be no longer used as an agent to combat the reddening on salted dried fish.

The effectiveness of sodium sorbate in preventing reddening and off-odour of salted dried fish as a result of *Halobacterium* growth was assessed and the result of bacterial growth are presented in Figure 9. Sodium sorbate for 1%, 2% and 3% treatments were 0.058%, 0.117% and 0.175%, respectively. Growth of the bacterium on SDF for 1% treatment increased slowly and the maximum viable count occurred on day 15. The counts were significantly lower than those of the control. Levels higher than 1% were not only bacteriostatic, but also bactericidal to the bacterium, since viable counts for 2% and 3% treatments were lower than the initial inoculum (3 log/g) within 4 days of incubation. Growth for these two treatments fluctuated from 3 to 4.5 log, throughout the incubation (18 days). Up to day 15 there was no evidence of reddening on the surface of SDF for 1% treatment, but a slight off-odour was recognized. The 2% and 3% treatment prevented the formation of off-odour in addition to preventing reddening. These results confirm the results on CHB and SDFH that sodium sorbate was effective against *Halobacterium sp*.

Sodium benzoate residues determined for 1%, 2% and 3% treatments approximated 0.0588%, 0.117% and 0.35% respectively. Viable counts for the three treatments decreased from 4 log/g to 3 log/g on day 2. Subsequently, this bacterium was not detectable until the end of incubation (day 18). In other words, salted dried fish dipped into 1% sodium benzoate solution contained no *Halobacterium* after 3 days of incubation. This suggests that the most effective preservative for preventing reddening in salted dried fish is sodium benzoate.

CONCLUSION

1. Against *Halobacterium sp.* the decreasing order of effectiveness was sodium benzoate, sodium sorbate, sodium propionate and sodium bisulphite.

2. At the highest level tested (0.03%) sodium bisulphite was not able to inhibit growth of *Halobacterium* in complex halophilic medium. Salted dried fish previously dipped in the highest level of 0.6% significantly reduced bacterial numbers, but reddening in SDF was not prevented. Therefore, this preservative cannot be recommended for preventing reddening on salted dried fish.

3. The use of sodium propionate at the highest level of 0.3% was not capable of inhibiting growth of *Halobacterium* in CHM, but could significantly inhibit growth in SDFH. Moreover, reddening in SDFH was not prevented. Sodium benzoate was most effective against *Halobacterium sp.* among the food preservatives tested. The use of 0.1% and 0.15% of this food preservatives in CHM prolonged the log phase for 5 and 6 days, respectively. In SDFH, the use of 1.5% was lethal to this bacterium which did not appear until the end of the incubation period. SDF previously dipped into a 1% solution and inoculated with 4 log/cm^3 was free from this bacterium.

4. Sodium sorbate was also active against *Halobacterium sp.* The use of 0.1% significantly inhibited growth in CHM, but did not delay the log phase, at the highest level tested of 0.2%. In contrast, the use of 0.1% in SDF prolonged the lag phase for 3 days and growth was still in the lag phase until the end of incubation when the concentration was increased to 0.15%. With SDF previously dipped in 1% of the preservative solution, this bacterium was completely inhibited, hence reddening in SDF was prevented.

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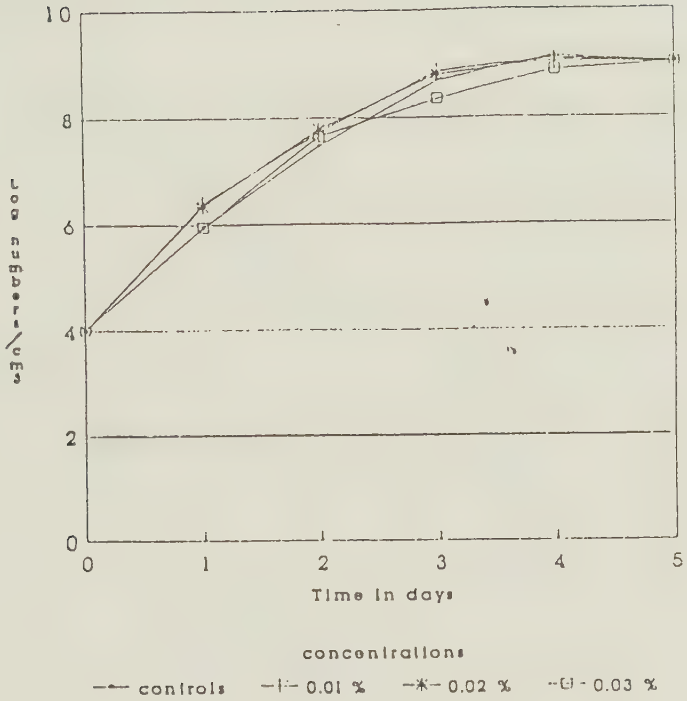

Figure 1. Growth of H. salinarium in CHB + sodium bisulphile

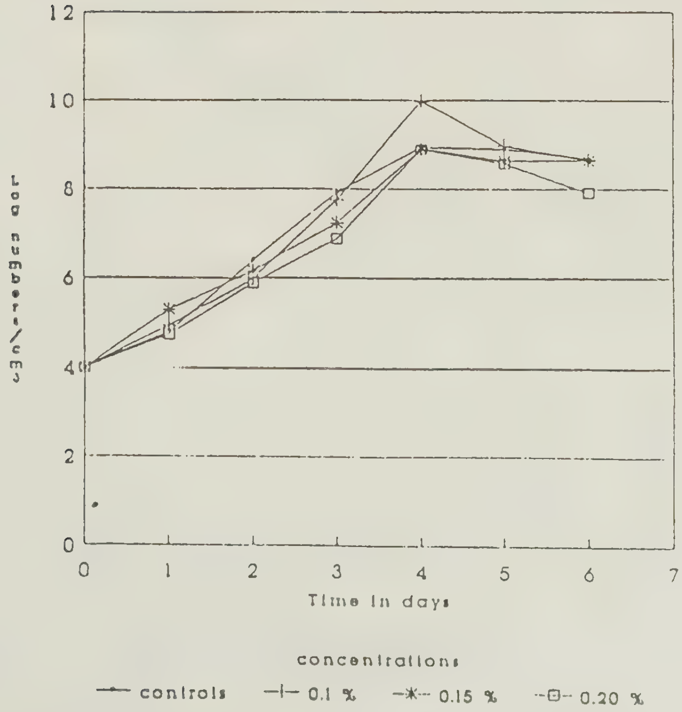

Figure 2. Growth of H. salinarium on CHB + sodium propionate

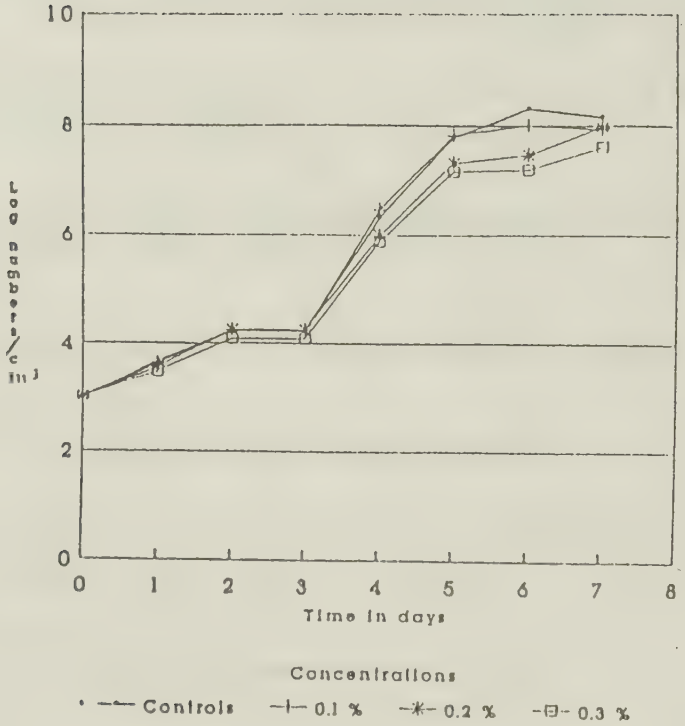

Figure 3. Growth of H. salinarium in SDFH + sodium propionate

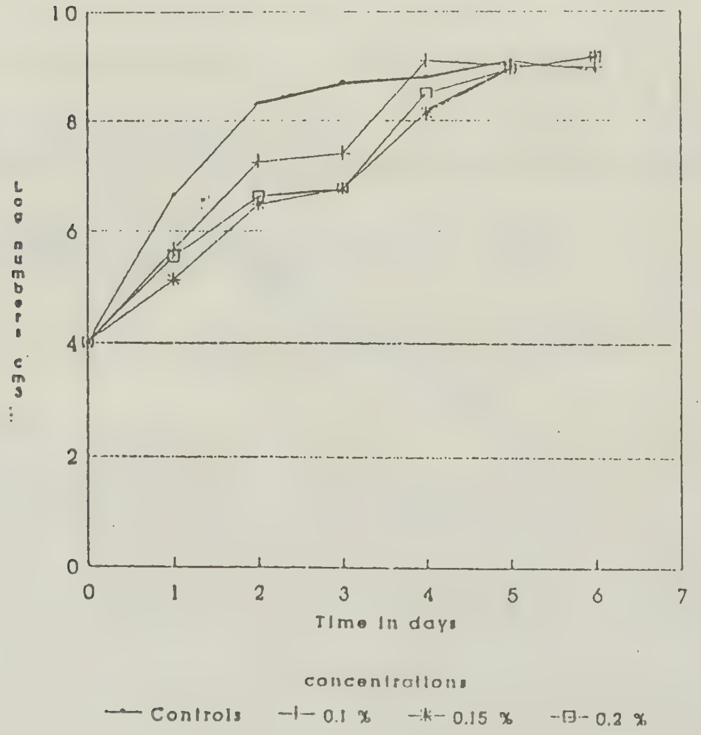

Figure 4. Growth of H. salinarium in CHB + sodium sorbate

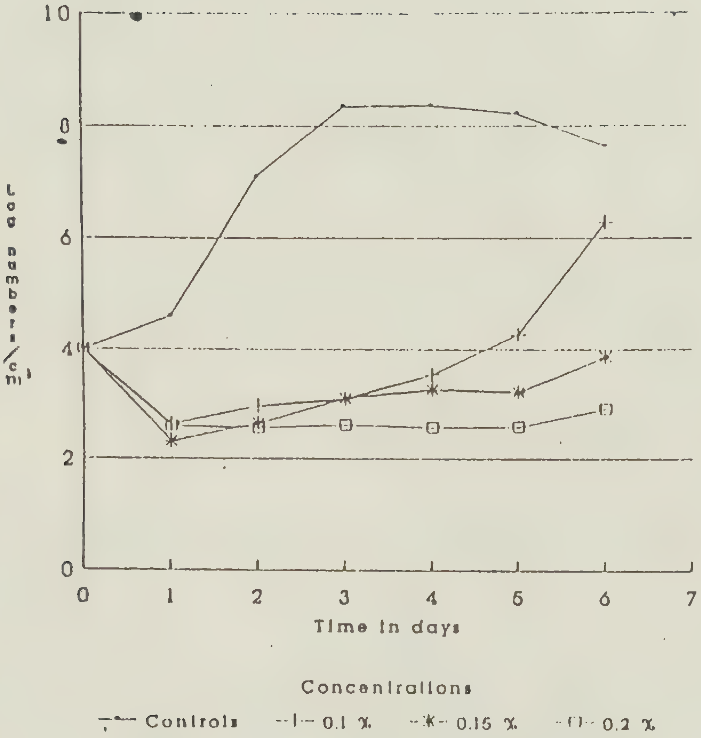

Figure 5. Growth of H. salinarium in SDFH + sodium sorbate

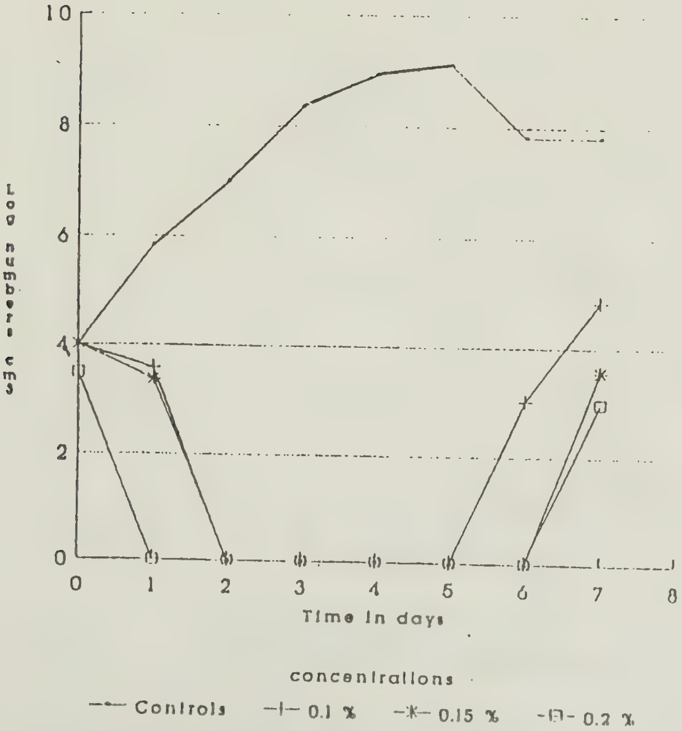

Figure 6. Growth of H. salinarium in CHB + sodium benzoate

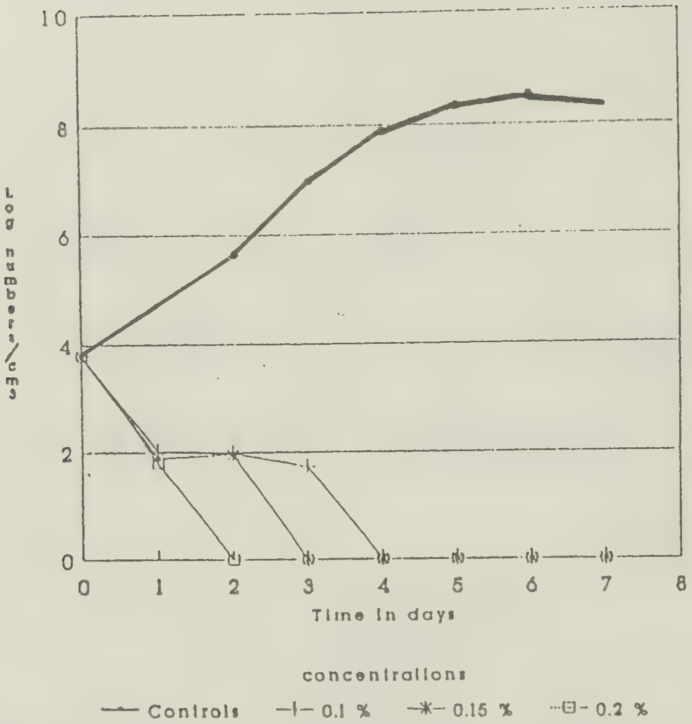

Figure 7. Growth of H. salinarium in SDFH + sodium benzoate

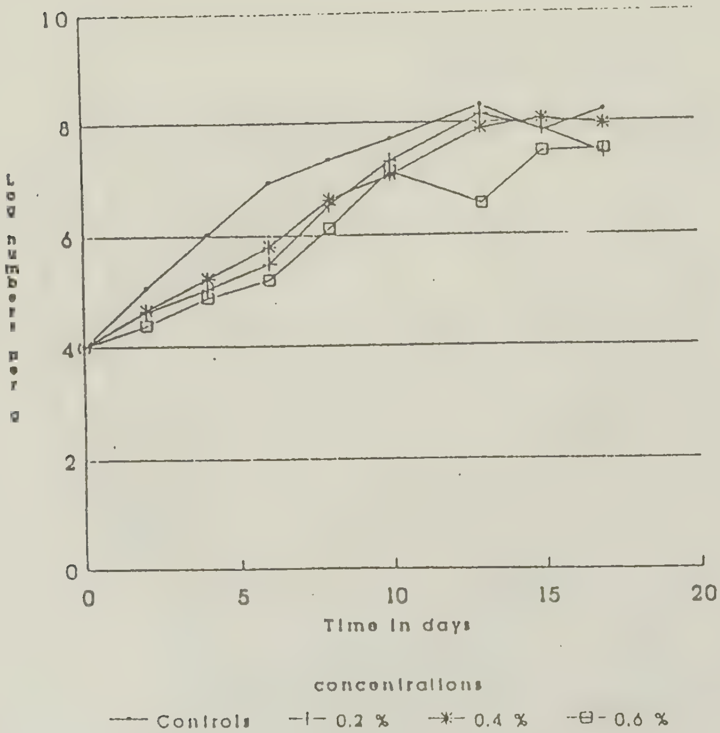

Figure 8. Growth of H. salinarium on SFD + sodium bisulphite

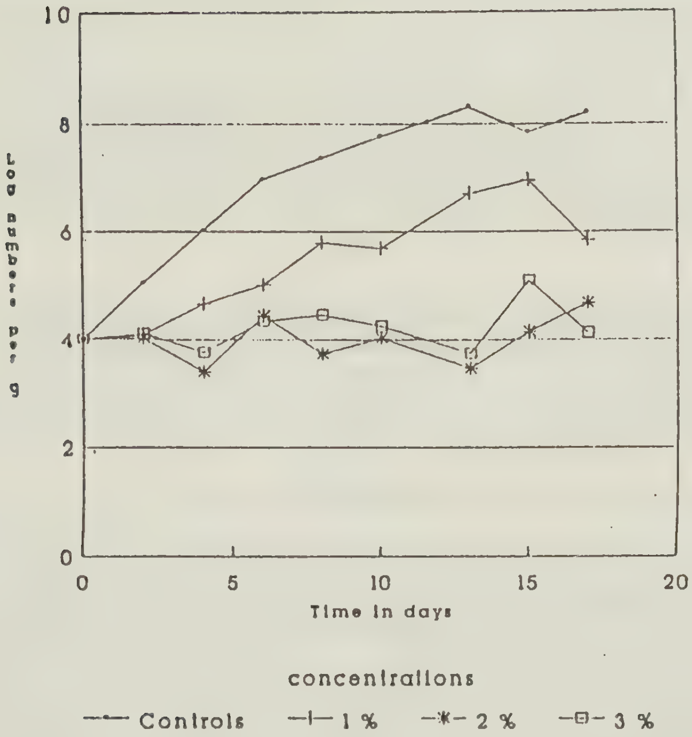

Figure 9. Growth of H. salinarium in SDF + sodium sorbate

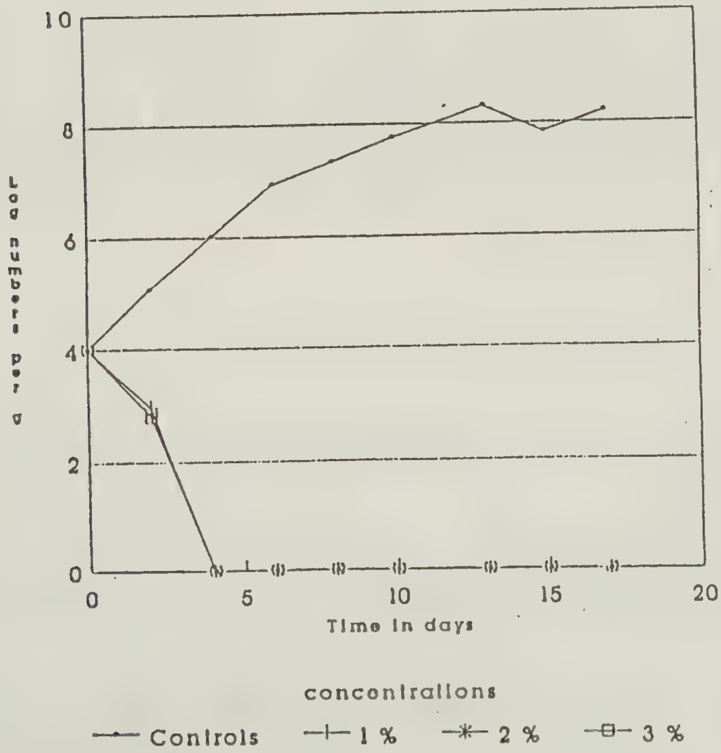

Figure 10. Growth of H. salinarium on SDF + sodium benzoate

REPROCESSING OF COMMERCIALLY CURED FISH FOR BETTER QUALITY AND SHELF LIFE

by

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ABSTRACT

The paper describes a process for the reprocessing of commercially available cured fish for improving its quality and shelf life. The method is a simpler modification of the earlier ones suggested by other workers, employing calcium propionate, an indigenously manufactured chemical. It consists of an initial refining of commercial cured fish by repeated washing in three changes of saturated brine, followed by dipping in a saturated solution of calcium propionate (approximately 5%) in saturated common salt, for a period of 2-5 minutes. The dip-treated fish is drained well and dried to bring down the moisture content to reasonable levels suitable for storage.

The method is equally applicable to freshly salted fish and is not species specific. The treated products are free from visible "red" and fungi for a reasonably long period (up to 6 months). They are also better in terms of sensory and bacteriological characteristics compared to the untreated controls. Insects are less attracted to the treated products packed in conventional types of containers like bamboo baskets, palmyra leaf baskets or gunny bags. The process is commercially feasible as proved by the experience of a few entrepreneurs who have prepared and sold at least 10 tonnes of refined and preservative treated cured fish packed in convenient retail packages. The cost of treatment of finished product is estimated to be Rs. 1,500/t. taking into account the present cost of the chemicals and labour involved.

INTRODUCTION

Considerable loss occurs during storage on account of infestation by red halophiles, fungi and insects in cured fish of commerce. Rancidity and brown discolouration are also major problems. Of these, reddening due to red halophilic bacteria is of greatest concern. There have been several attempts to find remedial measures for the control/prevention of this type of spoilage using chemical preservatives (Valsan, *et al. 1961,* Lahiry, *et al.* 1962); Rao and Valsan, 1962, 1962a, 1962b; Valsan 1963, 1963a). Valsan (1985) has described a process of treatment with calcium propionate to prevent red halophiles. The treatment consists of sprinkling calcium propionate on dry cured fish at the level of 0.3%. Alternatively dipping in 3% solution of the chemical in saturated brine has also been suggested. But the dipping process was less effective than the sprinkling process. Sprinkling of the chemical on large quantities of fish is laborious and do not ensure uniform distribution. Hence, it was considered worthwhile to devise a simpler, easily adoptable and commercially feasible method of treatment involving the same chemical. Simultaneously, sufficient care has also been taken to improve the general quality of the treated dry fish by a suitable modification of the process.

MATERIALS AND METHODS

Indigenously manufactured and commercially available calcium propionate (M/s. Industrial Organic Corporation, P.B. No. 7282, Bombay 77) was used in the experiments. The dip-liquor was saturated common salt solution, saturated with calcium propionate and containing 100 ppm available chlorine. Five percent calcium propionate was the maximum required. Butylated hydroxy anisole (BHA) at the level of 0.5% by weight of fish dissolved in alcohol was also added to the above mentioned bath when fatty fishes were dip-treated. The concentration of calcium propionate in the dip solution was retained by keeping the chemical packed in a cloth and squeezing it after every dip. Chlorine and BHA were replenished by measured addition after every dip.

The ratio of fish to dipping solution was 1:1.5 (wt:volume). Unless otherwise stated, commercially available dry cured silver belly, after a process of refining, was used for the experiments. The refining process consisted in washing the commercial dry cured fish in three changes of saturated brine to get rid of adhering sand, dirt, etc. In all cases, the dip-treated fish was drained well and dried in the sun. They were packed in polythene bags and stored in the laboratory. Observations were made at periodic intervals.

To arrive at the optimum duration of dip, trials were conducted employing dipping times ranging from instant dip to dip for 30 minutes.

The effect of different moisture levels in the finished products on the efficacy of the treatment was studied by drying the dip treated fish to different moisture levels.

The effectiveness of the dip treatment on different species of commercially available dry cured fish was studied with respect to shark, caranx, lactarius, oil sardine, lesser sardines, sole and mackerel.

The feasibility of re-using the same bath was also investigated. For this, serial dips were made in the same solution with replenishment of the chemicals after each dip as described earlier until the dipping bath became viscous and dirty.

Freshly salted tuna, shark, mackerel and oil sardine prepared in the laboratory were also dip-treated in the same preservation solution to study the additional shelf life on account of the treatment.

During storage, physical observations on colour, appearance, odour and incidence of "red" or fungi were made. Taste panel studies were carried out by experienced personnel to evaluate the organoleptic qualities of the products during storage. Counts of total halophiles and red halophiles were determined by using milk salt agar medium (Dussault and LaChance,1952). Standard Plate Count (SPC) and counts of coagulase + ve staphylococci, coliforms, *E. Coli* and faecal streptococci were determined by the methods described by Nair, *et al,* (1983).

RESULTS AND DISCUSSION

Details of storage studies on the samples dip-treated for different periods showed that dipping time of less than 2 min. was insufficient. Fish dipped for 2-5 min. exhibited almost identical shelf life of about 6 months, whereas the fish dipped for a longer time had similar storage life, but they were found to be disfigured in many cases; the peeling off of the skin being the most common manifestation. There was crumbling or powdering of the pieces after the treatment for more than 5 min. in some other cases. Hence the optimum dipping time was safely fixed as 2-5 min.

Study on the effect of moisture contents ranging from 33% to 48% in the finished products on the shelf life after the preservative treatment made it clear that even at 40% moisture level, the treated fish keeps well for a least 3 months. There is proportionate increase in shelf life with decreased moisture contents.

The results obtained on the treatment of different species of fish showed that all the species studied have extended shelf life because of the preservative treatment, whereas the untreated control samples spoilt within a couple of months. The treated samples remained in acceptable condition for more than six months.

The results on the storage life of the serially dipped samples did not show any difference in quality or shelf life with respect to the different samples, even among the first and last dipped ones. It was observed that there was accumulation of some dirt in the dip solution and the solution became viscous as the number of dips advanced. Such a solution could very well be filtered and reused after necessary replenishment.

It was very significant to note that none of the preservative treated samples developed visible "red" or fungi during a reasonably long period of storage. Further it was also observed that insects had much less attraction towards preservative treated fish even when packed in conventional type of containers like bamboo baskets, palmyra leaf baskets, or gunny bags. Many of the samples in such containers were insect-free for a long period compared to controls.

It was also observed during the course of the studies that standard plate count and counts of total halophiles and red halophiles registered substantial decreases as a result of the preservative treatment. Taking the typical example of commercially dry cured silver belly subjected to refining and preservative treatment, the values were as follows:

Untreated Control		Refined and Preservative treated
SPC	1.178 x 103	0.128 x 10^3
Total halophiles	1.281 x 10^3	0.452 x 10^3
Red halophiles	0.564 X 10^3	nil

Pathogenic organisms or organisms of public health significance were absent in all of the treated samples during storage.

The results of the trials on laboratory prepared samples also showed shelf life of more than six months indicating that the treatment is equally applicable to fresh fish immediately after salting just as in the case of commercially available salted/cured fish after refining.

The process is easily adaptable on a commercial scale and opens up a new avenue in dry fish preservation in India. The commercially cured fish prepared at different small units scattered all along the coast line can be brought to a few central places where it can be subjected to the process of refining and preservative treatment. This will improve the quality and shelf life of the product considerably. The refined and treated products are far more attractive and appealing compared to the conventional commercial ones, especially when presented in convenient retail packages. The treated products have much longer shelf life so that they can be kept in godowns awaiting sale until the prices become profitable. The cost of treatment at the current market price of the chemicals used and the labour charges involved in Rs. 1,500/- per tonne of the finished products. Compared to the benefits the additional cost is insignificant.

The commercial feasibility of the process has been established by a few entrepreneurs of the region who have already prepared and sold not less than 10 tonnes of refined and preservative treated dry cured fish belonging to various species under different brand names. All of them have reported good sales and reasonably high profit.

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PROCESSING OF DRIED SALTED RAY AT MUARA ANGKE

by

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ABSTRACT

A study on the processing of dried salted ray at Muara Angke was carried out. Three processing units were chosen for observation of all technological aspects including raw material, processing methods, equipment, conditions of sanitation and specification of end product.

The result of the observation showed that materials used were of low quality and the processing method was also poor, resulting in low quality products. Additionally, waste produced from dried salted ray processing was high and it was not treated or utilized properly, creating pollution problems especially around processing area.

INTRODUCTION

Ray is a demersal fish found in almost all Indonesian waters. The total catch is high, reaching 18,974 tons with the value of 5,785 million rupiahs (Anon, 1987). Although the production of ray is high, the price is relatively cheap, while the utilization is limited. This is because of the ammoniacal flavour of the meat, making it objectionable to consumers.

Ray is only processing traditionally as dried salted fish and a little is consumed as fresh fish or smoked fish. The skin is sometimes processed into shark-fin like fibre. A study on the use of frozen surimi from ray has been conducted to decrease the ammonia flavour and to improve its utilization (Nasran *et al.*, 1988), as well as on the tanning of ray skin into a high quality leather (Haq *et al.*, 1990).

In Indonesia, dried salted ray has a good market especially in West Java. High quality dried salted ray commonly gets a high price in the market. The quality of dried salted ray is affected by species and freshness of raw material, as well as processing method and sanitation.

To improve the quality of dried salted ray and the value/price, it is necessary to have complete data and information on the processing. Observation of dried salted ray processing and end product quality in this report, was carried out in Muara Angke, Jakarta, a centre of traditional fish processing controlled by the Government.

METHODS

To achieve an objective picture of dried salted ray processing, three processing units (A,B,C) were chosen, assuming that they represent the population of processors in the area.

In this study, observation was made of all technological aspects such as raw material, processing methods, equipment, condition of sanitation and specification of end product.

Analysis also included:

a. Raw material: species, size and freshness (organoleptic, chemically and microbiologically)

b. Salt: type, quality (organoleptic, NaCl content, impurities and halophilic bacteria)

c. Processing water quality: physical quality

d. Processing method, including packing, equipment, sanitation, and intensity of fly infestation

e. Product specification during processing (organoleptic, chemical and microbiological)

f. End product specification.

RESULTS AND DISCUSSIONS

The general process flow of the processors at Muara Angke is as shown in Figure 1.

Raw material

Raw material used in this study was landed in Muara Angke, Jakarta. The catch was from a wooden bottom long liner (20-30 gross tonnes) equipped with fishhold and the fish was preserved with ice.

Fishing was East of Sumatra and South of Kalimantan for 20-30 days. The catch composition was commonly heterogenous both in species and size. Species of ray usually caught are *Actobatus nari-nari, Tainiura melanospila, Dasyatis ushici, Himantura narnak, Gymnura japonica, Rhinoptera japonica* and *Dasyatis akajei.*

While waiting for unloading, the fish was kept in the hold for 1-2 days. The size of ray used in this study was varied, i.e., 2.5-43 kg, while the specification of rays used in this experiment is as shown in Table 1.

Table 1 showed that freshness of raw material used by processor B was better than that used by the other processors in relation to the organoleptic score and lower TVB, pH and TPC values. The freshness of raw material used by processor B might be due to the shorter catching operation (five days trip), while raw material used by the other processors originated from bottom long liners which usually take longer trips, up to one month.

Table 1. Specification of ray used as raw material for dried salted ray

Parameters	Processing units		
	A	B	C
Organ.quality:			
score (10 scale)	4.0-7.0	7.5-8.0	3.5-4.0
Description	pinkish white sl. dark brown, sl.dull, fishy & strong stench odour, soft & loose, sl. juicy	reddish white, sl. dull, sl.fishy, soft urea odour, firm & elastic	pink, dark-brown, dirty, fishy & strong ammonia odour, soft & loose, juicy
Chem.quality:			
moisture (% wb)	76.14-78.73	78.04-79.53	81.06-81.25
ash (% wb)	1.30-1.49	1.31-1.44	1.63-1.67
protein (% wb)	10.46-13.85	13.31-14.91	12.40-13.15
fat (% wb)	0.90-1.21	2.41-2.48	0.62-0.72
NaCl (% wb)	0.58-1.66	0.40-0.86	0.45-0.82
a_w	0.95	0.94-0.95	0.97-0.98
TVB (mg % N)	115.85-218.40	42.27-48.98	567.53-579.27
NH_3 (mg % N)	0.42-0.52	0.54-0.57	0.82
pH	7.10-8.60	6.64-7.63	8.79-8.81
Micro.quality:			
TPC	8.3x10^{5}- 990x10^{5}	1.8x10^{5}- 1.9x10^{5}	1.3x10^{6}- 1.8x10^{6}
Fly attack	very little	none	little

The strong ammonia smell of ray is due to the urea content found in the meat. During deterioration urea is converted into ammonia, therefore the lower quality of ray, the stronger the ammonia smell is.

Additional material

Salt

Salt used by processors is solar salt with coarse crystals. This salt was bought from cooperatives at the price of Rp 105,- per kilogram and packed in plastic bags of 50-80 kg weight. The quality of salt is presented in Table 2.

Purity of solar salt used by processors was low. This, of course, affected the salting process by inhibiting NaCl penetration. Consequently, the quality of end products was also affected where the appearance was bad, the texture was tough and the flavour was bitter.

Halophilic bacteria present in common salt, also affected the end product. When storage conditions were not adequate, the bacteria will grow rapidly decreasing quality and economic value.

Processing water

Water used for processing of dried salted ray, originated from brackishwater ponds located around the processing area. The appearance of this water was slightly muddy (keruh) and the colour was yellowish/greenish. This water was used directly for processing without being treated.

Based on sanitation and hygienic considerations the use of brackishwater without treatment is not recommended.

Processing methods

Preparation of raw material

The ray was unloaded and weighed in the auction place, then transported to processing location which took 10 minutes by pushcart, without ice. Upon arrival at the processing location, the ray was piled on the processing floor, which was dirty, waiting for processing.

The fish was then dressed and cut with a long thin blade into parts (wing meat and waste, such as head, backbone, viscera, level and tail) as shown in Figure 2. For certain species of ray *(Dasyatis akajei, D. ushici)* which was considered high quality, the back was first deskinned and then the rest was dressed. This skin was sold at a price of 800-1,000 rupiahs, to be processed into tanned skin or shark-fin like fibre.

Table 2. Salt quality used for dried salted ray

Parameters	Processing units		
	A	B	C
Organ.quality:	brownish, dark, dirty, small crystal, ammonia smell	white, sl.dirty, sl.big crystal, fishy smell	white, dirty dark, small crystal, more foreign matter
Chem.quality:	76.85	72.62	76.81
NaCl (%)			
Moisture			
Impurities	0.1125	0.1364	0.1363
Mg (ppm)	0.0052	0.0050	0.0052
Fe (ppm)	0.0142	0.0161	0.0166
Ca (ppm)	0.0016	0.0017	0.0016
Pb (ppm)	1.52	0.82	0.92
dirt (%)			
Micro.quality:	7.9×10^3	5.5×10^3	3.8×10^3
Hall. bact.			

The dressing and weight loss of fish during preparation of raw material is presented in Table 4.

During preparation, fly attack seemed to be high in processing unit C where the sanitation condition and raw material quality were poor (Table 1). On the other hand, preparation in processing unit B was almost free from fly attack, due to the high sanitation standards and raw material quality. It is clear that a dirty working area and the poor quality of raw material promoted stale odours that attract flies.

In dried salted ray processing, only wing meat is processed. The percentage of this part was in the range of 65-80% depending upon species and size of fish and the cutting method, while the other part (20-35%) was wasted. At present, the latter part is disposed of on open land without any treatment. It creates environmental problems and needs to be properly utilized and treated.

Table 3. The dressing condition and weight decreasing of fish during preparation of raw material

Parameters	Processing units		
	A	B	C
Sanitation condition	bad	good	fair
Fly attack	****	**	***
Weight composition of cut fish			
small size			
whole fish (kg)	2.5 (100%)	4.2 (100%)	10.0 (100%)
wing meat (kg)	1.8 (72%)	2.8 (66%)	7.0 (70%)
waste (kg)	0.7 (28%)	1.5 (34%)	3.0 (30%)
medium size			
whole fish (kg)	7.6 (100%)	12.5 (100%)	13.0 (100%)
wing meat (kg)	5.6 (74%)	8.8 (70%)	9.5 (73%)
waste (kg)	2.0 (26%)	3.7 (30%)	3.5 (27%)
large size			
whole fish (kg)	43.0 (100%)	16.5 (100%)	17.0 (100%)
wing meat (kg)	33.6 (78%)	11.0 (67%)	12.5 (74%)
waste (kg)	9.4 (22%)	5.5 (33%)	4.0 (26%)

Salting method

Before being salted, the wing meat was washed with brackish water in a wooden basin. During washing, the water was not changed, so that the appearance of water was dirty due to blood, slime content, etc. The washed meat was then salted (pickled) in a water-tight wooden basin with salt on the bottom, salt was poured over the meat in layers. The top layer of meat was covered by dense salt. There was no weight on the top layer. This was done at ambient temperature with salting time of 31-32 hours.

The percentage of salt used by each processor varied greatly, i.e., 17% for processor B, 28% for processor C and 39% for processor A. The more salt used by both processor A and C seemed to be due to the low quality of raw materials. The use of more salt is to lower the rate of spoilage during the salting and drying process. Processor B used less salt because of the better quality of raw material and to lower the salt content of the end product. During the salting process, the fish was almost free from fly attack because the top of the layer was covered with salt (Table 4).

A salting process of 31-32 hours seemed insufficient to allow complete salt penetration into the fish muscle. Moreover, in the salting process observed, the processor used solar salt, which had low quality (Table 2), so that the salt penetration went slowly. It was shown that the salted rays still had the same soft/loose texture and reddish colour as fresh fish. Since the salt penetration went slowly, it caused deterioration as shown in Table 4, where the TVB, NH_3 and pH of salted rays is high due to the activity of enzymes and/or bacteria.

Table 4. Meat condition after salting

Parameters	Processing units		
	A	B	C
Organ.quality:			
Description:	grey creamy, dull, v. dirty, ammoniacal odour, sl. loose, juicy, sl. soft	white creamy, dull, sl.dirty, fishy odour, sl.loose	reddish cream, sl.brownish, fishy & ammoniacal odour sl.strong, sl.loose
Chem.quality:			
Moisture (%)	68.07-73.51	67.62-68.51	69.57-69.59
Ash (%)	6.70-10.12	9.70-10.00	9.55-10.86
Salt (%)	4.99-7.01	7.91-8.00	8.27-8.96
TVB (mg% N)	289.63-388.27	299.06-238.75	407.89-408.0
pH	7.97-8.28	7.93-8.02	8.83-8.87
a_w	0.91-0.92	0.89	0.91-0.92
Microb.quality:			
TPC	4.8-3400x10^5	56-330x10^5	6.5-520x10^5
MHA bacteria	1.3-20.1x10^5	29.9-72x10^5	1.21-380x10^5

The condition of brine formed during the salting process also showed that spoilage occurred during this time. The brine was dirty and had a strong ammoniacal stale odour, especially processors A and C (Table 5).

Table 5. Brine condition used for salting process

Parameters	Processing units		
	A	B	C
NaCl (%)	25.81	26.35	25.81
Total solid (%)	0.51	0.60	0.21
Organ.quality:			
Description:	dark brown colour, very opaque, dirty, lot of sediment, strong ammoniacal odour almost stale	brown colour, opaque, sl. sediment, fishy odour, sl. ammoniacal odour	reddish brown colour, sl. sediment, ammoniacal odour sl. strong

Although the NaCl content of the brine was rather high, the salt used was of low quality, which might inhibit salt penetration so that the rate of spoilage became faster.

Filleting process

Salted fish was unloaded from the wooden basin, washed with brine formed during salting and placed on the processing floor.

Filleting was done using a sharp thin blade. The process was done between midnight and dawn after the salting process finished.

Based on the number of workers, filleting speed and fish (Table 7), it can be seen that the filleting process was done by skilled labour. Since this process was done at night, it was free from fly attack. The size and thickness of fillets varied due to the sharpness of blade, labour skill and texture of salted meat. The length of fillet is 14-55 cm, with the width of 8-37 cm and a thickness of 3-12 mm. Thin dried salted ray is usually preferred by consumers.

Drying process

Filleted meat was washed with a mixture of 3-4 part of brackish water and 1 part of used brine. This water had a salt concentration of 6%. Washing was by immersing the meat several times in the water, and then draining before being dried.

Drying was done in the sun with the filleted meat placed on a raised bamboo tray approximately 1 metre above the ground. During drying, the meat was turned over twice to ensure even drying.

Table 6. Technical condition during drying

Parameters	Processing units		
	A	B	C
Air temp. (°C)	28.38	26.36	26.5-39
Relative humidity	32-67	49-82	50-77
Weather	hot, windy	hot, windy	cloudy, strong wind
Sanitation	very dirty	clean	fairly clean
Fly attack	lot	little	little
Drying time (hrs)	7	8	8
Weight loss during drying (kg)			
after 2 hrs	0.73	0.20-0.35	0.10-0.30
after 4 hrs	1.03-1.49	0.35-0.60	0.30-0.40
after 6 hrs	0.54-0.90	0.25-0.35	0.25-0.30
after 8 hrs	finished	0.10-0.20	0.10-0.15

Since fat content of ray was low, (0.62 - 2.48% - Table 1) and the fillet was thin, the drying process did not meet many problems. The drying time was relatively short due to the weather condition (hot and strong wind), and almost free from fly attack. However, salted ray processed from bad quality fish as well as thick fillets attracted flies because of stale odours (Table 6).

Packing technique

Dried salted ray was piled on bamboo trays (thin woven bamboo slats) or gunny and kept for 30-60 minutes waiting for grading. The dried salted ray was graded based on the species of fish or the colour of meat and then packed in bamboo baskets of 40-60 kg weight. The dried salted ray was arranged with the sharp end pointed to the centre position. The smaller and bad quality products were arranged on the bottom, while the big sized and good quality products were placed on the top of the pack. Sometimes, to get compact packing, the top of dried salted ray was stepped on. The top of dried salted ray was then covered by cement sacks or used newspaper and then tied with plastic rope.

The packing was done by 3-5 persons for one hour in the packing room which was free from fly attack. This room was open, separated from the processing area and used as storage room (warehouse) before marketing.

Yield

The yield of dried salted ray processing was low and varied among the processors. The highest yield was by processor B (30%), while processor A and C produced lower yields, i.e., 20% and 21% respectively. This condition seemed to be due to the freshness of raw material, size and species of fish, dressing and filleting.

End product specification

In general, the specification of dried salted ray is shown in Table 7.

Organoleptically, the dried salted ray produced by the three processors was not much different. The high moisture content facilitated bacteria and enzyme activity resulting in increasing spoilage compounds such as TVB and NH_3 which affect the smell. However, from the chemical quality (NH_3, TVB, peroxide and a_w value), the dried salted ray produced by processor B was better compared than the others. This is because raw material used by this processor was better. Additionally, the sanitation conditions of processor B were better.

Table 7. Specification of dried salted ray

Parameters	Processing units		
	A	B	C
Organ. quality			
score (63 scale)	47.90	53.75	51.05
description	clean, sl.physic. damage, thick, light-dark brown, strong ammonia odour, firm, humid	less clean, thick, sl.dull, reddish brown, ammonia odour, loose, humid	dirty, sl.physic, damage, salt crystal appeared, thick, sl.dull, light dark brown, strong ammonia odour compact, humid
Chem. quality			
moisture (%wb)	40.65-48.19	38.82-40.19	40.72-42.37
ash (%wb)	12.92-19.82	16.28-18.63	19.67-21.90
protein (%wb)	22.93-59.86	38.31-41.28	32.27-38.02
fat (%wb)	0.78-1.27	1.03-1.17	0.86-0.95
salt (%wb)	9.96-14.71	11.05-11.22	16.53-17.53
NH_3 (mg% N)	0.77-0.95	0.42	0.77-0.83
TVB (mg% N)	81.50-410.57	273.80-273.98	317.85-335.08
Peroxide (%)	83.50-200.98	13.29-25.69	251.57-257.92
a_w	0.72-0.81	0.71	0.71-0.72
Microb. quality			
TPC	$3.9\text{-}204 \times 10^5$	$3.2\text{-}31 \times 10^5$	$8.7\text{-}15 \times 10^5$
MHA bacteria	$1.2\text{-}47.8 \times 10^4$	$7.1\text{-}10.3 \times 10^4$	$4.3\text{-}21.3 \times 10^4$

CONCLUSIONS AND RECOMMENDATIONS

From the observation of dried salted ray processing at Muara Angke, Jakarta, it can be concluded that:

1. Materials used including fresh ray, salt and water have low quality which results in low quality end products.

2. Processing method was not adequate, including transportation of raw material, dressing, salting, filleting and packing, resulting in low quality end products.

3. Waste produced from dried salted ray processing was considerable and was not treated or utilized properly, so that it created pollution problems especially around processing areas.

To improve the quality of dried salted ray produced by processors at Muara Angke, Jakarta, it is recommended to:

1. Improve raw material quality by proper handling started from catching and landing. When possible, dressing and/or salting is done on board.

2. Use of clean and covered wooden boxes for rapid unloading to avoid prolonged exposure to sunlight.

3. Use better salt quality for salting and clean water which meets drinking water standards.

4. Improve filleting and packing techniques.

5. Use of dryer for faster drying especially for bigger size of fish and thicker fillets.

6. Keep the waste (including headbone, backbone, viscera and tail) away from fish which will be processed.

7. Treat and utilize waste produced from dried salted ray by processing it into meal, silage, fertilizer, glue, etc.

ACKNOWLEDGEMENT

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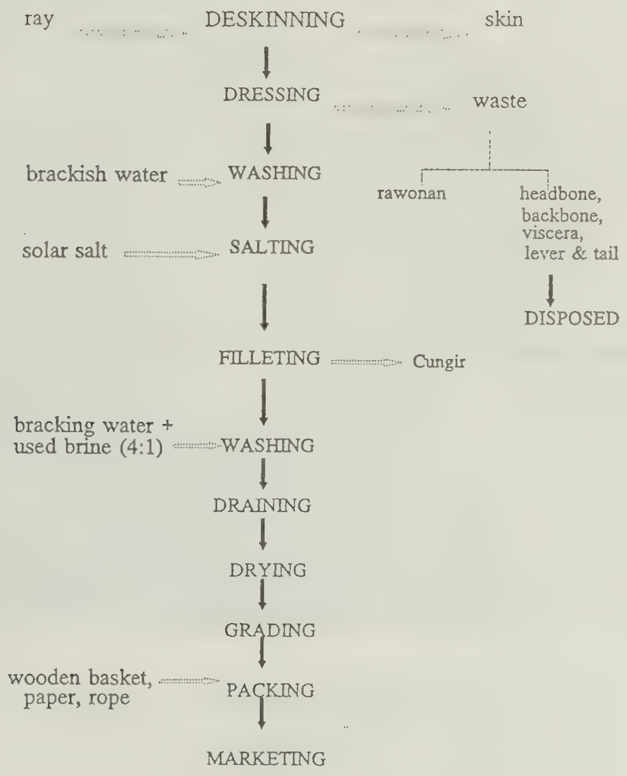
ray
DESKINNING
skin
DRESSING
waste
brackish water
WASHING
rawonan
headbone,
backbone,
viscera,
lever & tail
solar salt
SALTING
DISPOSED
FILLETING
Cungir
bracking water +
used brine (4:1)
WASHING
DRAINING
DRYING
GRADING
wooden basket,
paper, rope
PACKING
MARKETING

Figure 1. Process flow

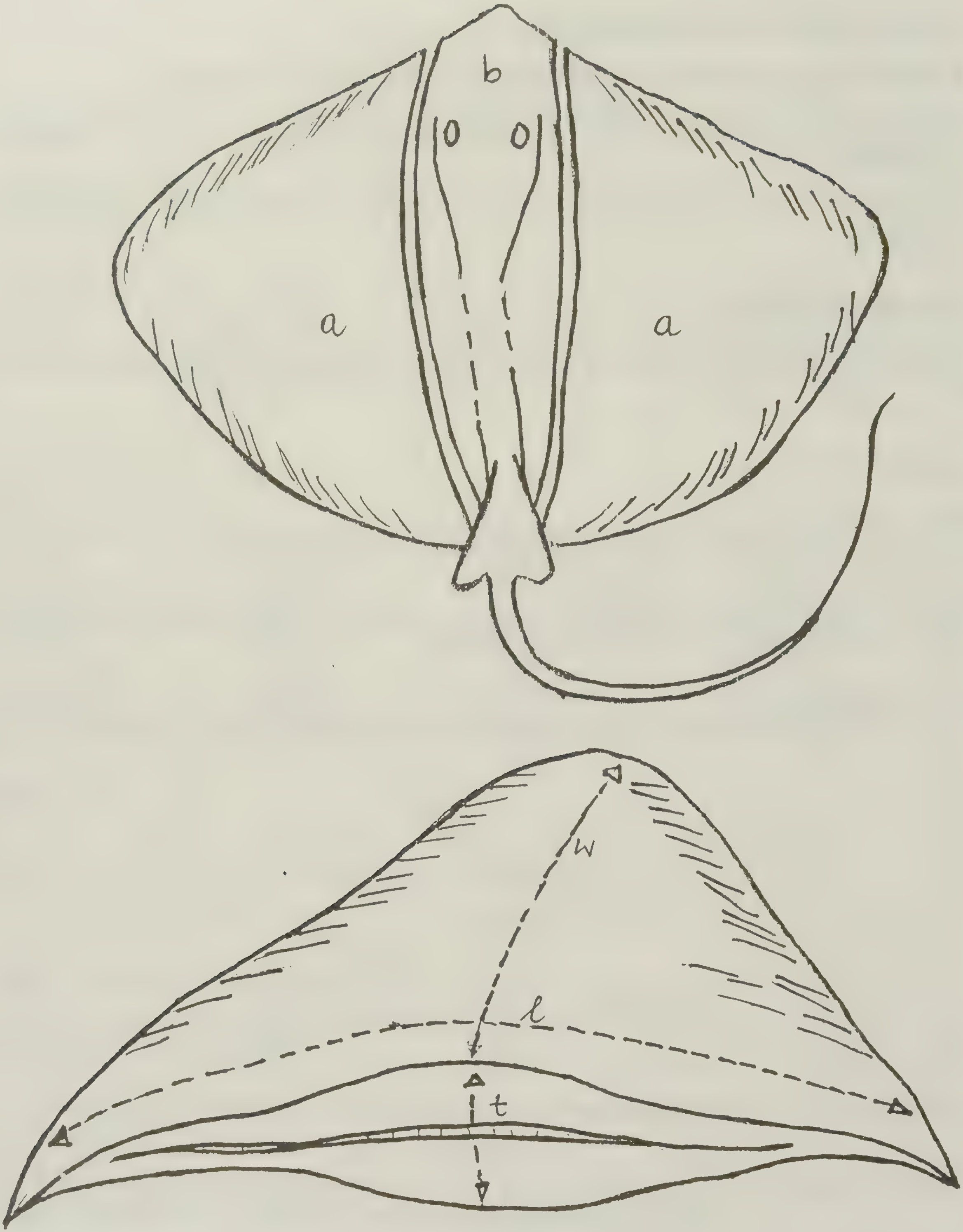

Figure 2. Dressing and cutting technique of raw ray

a : wing slices
b : waste (head, backbone, viscera, lever, tail)
l : length of wing slice
w : width of wing slice
t : thickness of wing slice

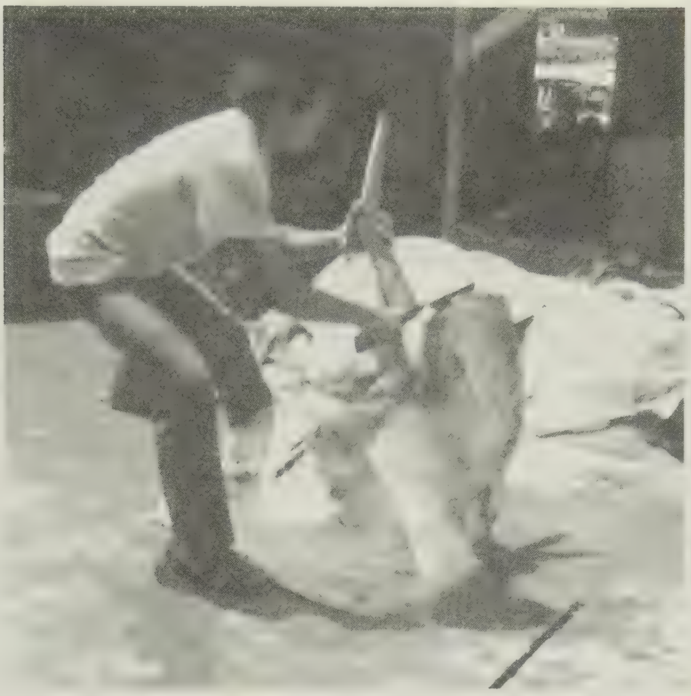

Figure 3. Dressing technique of raw ray

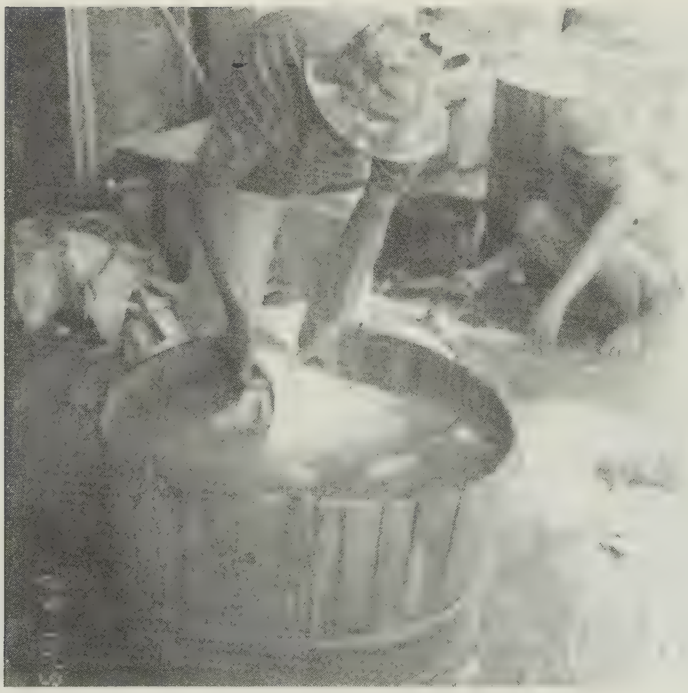

Figure 4. Washing of wing slices

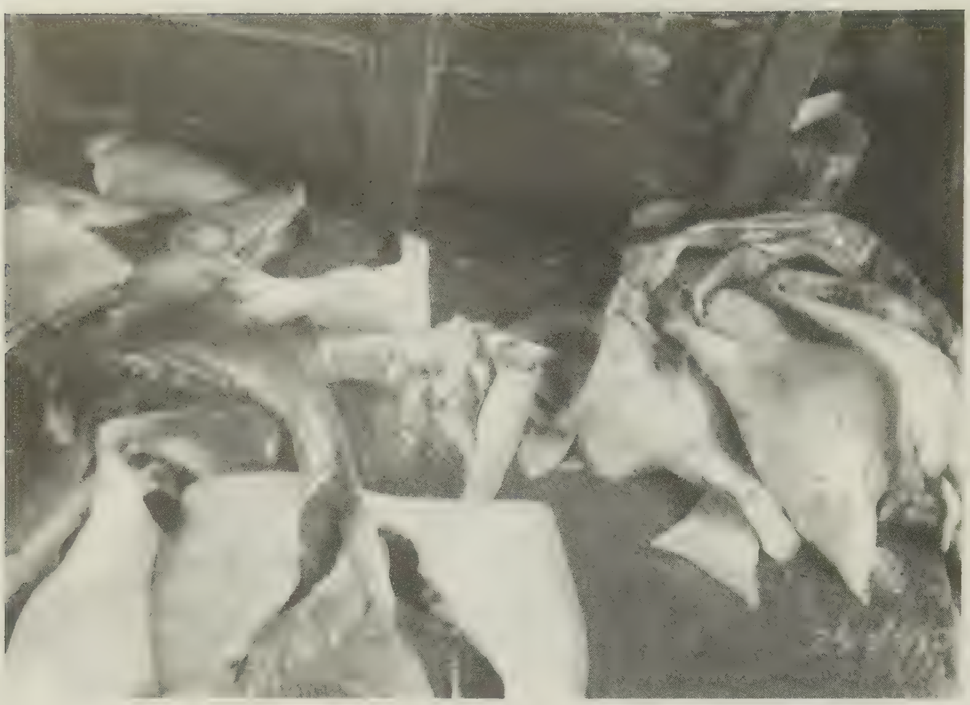

Figure 5. Washed meat ready to salt

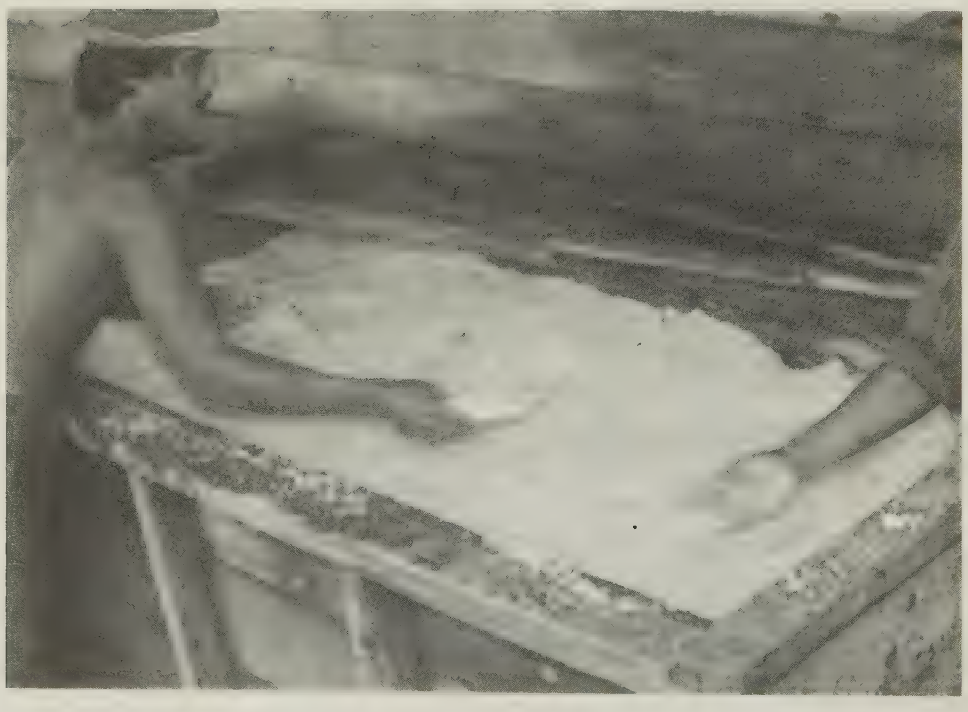

Figure 6. Salting process of ray

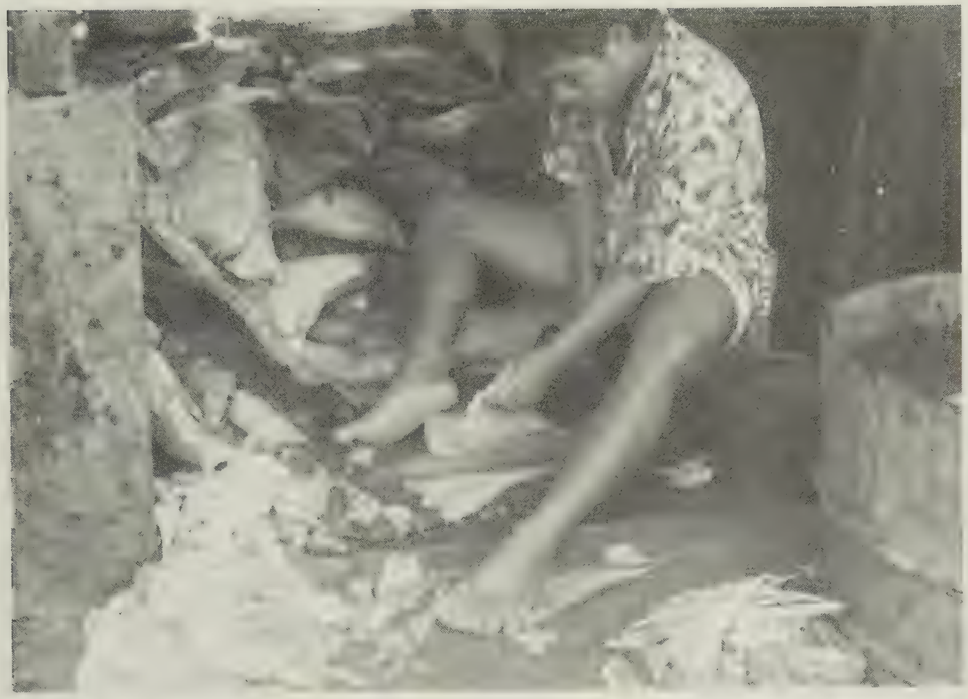

Figure 7. Filleting of salted ray

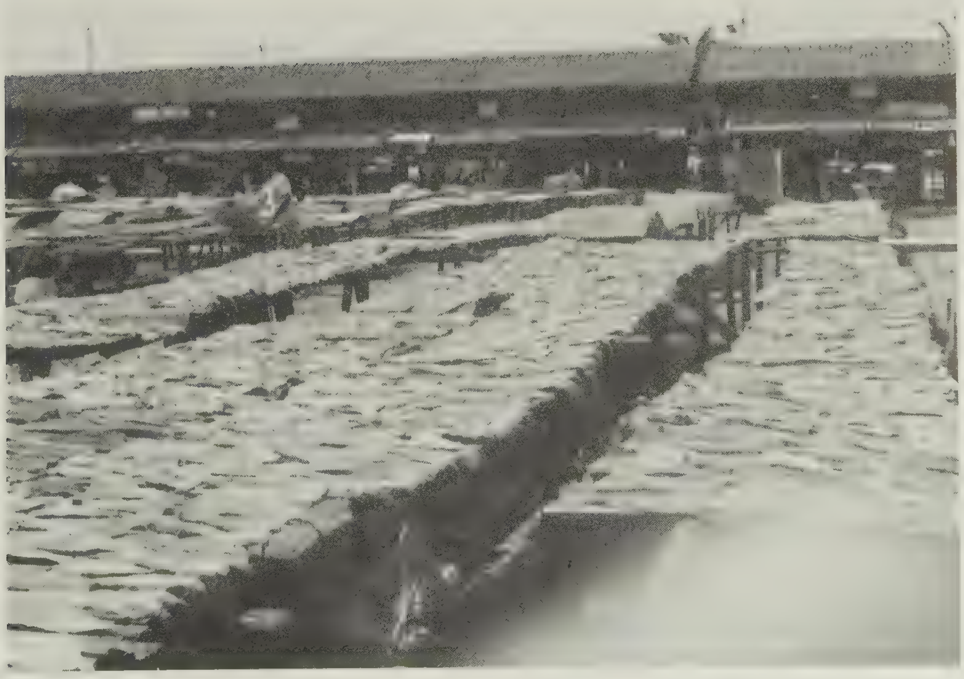

Figure 8. Sun drying of salted ray

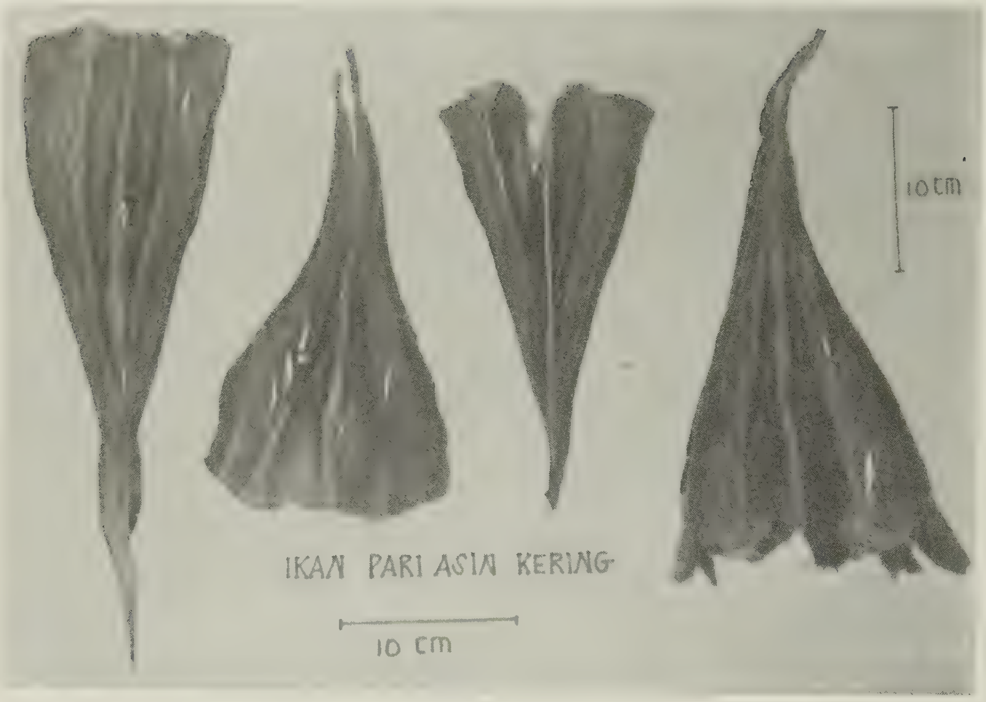
10cm
IKAN PARI ASIN KERING
10 cm

Figure 9. Dried salted ray

WATER ACTIVITY AND SHELF-LIFE OF SEMI-DRIED SALTED SHARK FILLETS

by

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ABSTRACT

Three batches of semi-dried salted shark fillets having moisture contents between 54 and 61% and salt contents between 19 and 23% were equilibrated at different water activities (a_w). Their storage and sorption behaviour under these conditions were studied to estimate the shelf-life and quality problems associated at different a_w's.

Samples stored at a_w of 0.85 and above absorbed moisture whereas the samples stored at 0.65 and below showed drastic reduction in moisture contents and hence weights. The samples stored at a_w of 0.75 had retained the consistency of the product to the maximum extent. The important physical changes noticed in samples stored at a_w of 0.85 and above were softening of muscle and yellow discolouration. Between a_w of 0.50 and 0.85, fungal colonies were also noticed. Red discolouration was noticed in samples stored above a_w of 0.43. A direct co-relation was observed between water activity and bacterial growth. The isohalic sorption isotherms for semi-dried salted shark fillets were similar to those for other salted and dried fish reported earlier. Water activities of the salted fillet at different constant relative humidites were calculated from the sorption data. The average monolayer value of semi-dried salted shark fillet was 5.33 gm/100 gm of solids.

INTRODUCTION

The main objective of any seafood preservation process is to extend the shelf-life of the products and to allow maximum storage period and convenient distribution. The activity of micro-organisms is one of the most difficult problems which limit the shelf-life of the product. Salt curing is one of the methods to control the growth and activities of micro-organisms by bringing down the water activity. Salting will increase the osmotic pressure and hence reduce the availability of water for microbial growth.

By plotting a_w value as a function of the amount of water present, a schematic representation is obtained which indicates the manner in which water is bound or unbound. This construction on the storage behaviour and quality changes at different a_w is essential to decide the method and duration of salting and drying. It is also very important to adopt proper packaging and storage practices for the products. Karel (1975) described various methods to estimate a_w and sorption isotherms in determining the stability of foods during storage. Little is known about the storage behaviour of seafoods prepared from fish, especially of tropical marine fish, at different a_w. Doe *et al.* (1982) determined isohalic sorption isotherms for dried salted cod *(Gadus morrhua)* and provided a method for calculating a_w of salted fish product from its moisture and salt contents. Poulter, Doe and Olley (1982) used the isohalic sorption isotherms of cod in conjunction with growth data for dun mould (*Wallemia sebi*) to predict the shelf-life of dried salted fish in tropical climates. Curran and Poulter (1983) also worked on the sorption behaviour of dried salted lisa (*Xenomugil thoburni*) produced under semi-commercial conditions. Their results confirmed that the procedure for predicting the a_w of cod muscle from the physical properties of sodium chloride solution and the sorption characteristics of fish muscle can be applied to tropical fish processed commercially.

Here an attempt has been made to study the shelf-life storage and sorption behaviour of three batches of semi-dried salted shark fillets having moisture contents ranging between 54 and 62% under different constant a_w. The a_w of the fillets were worked out from their salt, moisture and fat contents.

MATERIALS AND METHODS

Three batches of shark (B_1, B_2, B_3) of species *Carcharinus melanopterus* and size range between 75 cm and 81 cm were used for the study. The semi-dried salted fillets were prepared as per the method described by Ramachandran and Solanki (1988). The fillets (2.5 cm x 1.25 cm x 7.5 cm) thus prepared were equilibrated under different constant a_w as described by Karel (1975). The experiments were conducted at room temperature (25 ± 3°C). Some samples were also kept in open condition without any a_w control.

The physical and olfactory observations on the appearance, colour, odour, texture, presence of visible bacterial and fungal colonies were periodically carried out during storage. The test panel score for the physical characteristics of the fillets during storage were noted. The weight gained/lost during storage under different constant relative humidity conditions were also noted. The moisture, salt and fat were estimated by the method of AOAC (1975). Nitrogen was estimated by the Micro-Kjeldahl method. The bacteriological tests were carried out by the standard plate count method. The Total Viable Count of bacteria was estimated by using Tryptone Glucose Agar, Faecal Streptococci count by K.F. Agar, *Escherichia coli* count with Tergitol 7 Agar and Coagulase Positive Staphylococci using Baird Parker Agar. Incubation was done at 37°C. The Total Fungi Count was estimated using Czapek's medium and incubated at room temperature (25 ± 3°C) as per standard spread plate method described by Pitt and Hocking (1985).

The periodical observation on the gain/loss in weight of the fillets was noted until the samples attained equilibrium. After attaining equilibrium, their moisture and salt contents were estimated. From the values of moisture, salt and fat content at the time of equilibrium, sorption data and isohalic isotherms were worked out as described by Curran and Poulter (1983). Water activity of the fillets were worked out as described by Doe (1986). The monolayer value of salted semi-dried fillets were calculated by BET (Brunauer-Emmet-Teller) Isotherms as described by Karel (1975).

RESULTS AND DISCUSSION

The proximate composition of the three batches of semi-dried salted shark fillets are given in Table 1. The fillets had a moisture content in the range of 54 to 61% and salt content between 19 and 23%. The fat content was negligible.

The changes in the weight of the fillets during storage under different constant a_w are given in Figures 1 to 3. The samples (B3) were an initial moisture level of 54% and salt of 23% and stored at an a_w of 0.75 retained its weight with negligible loss (about 1%). The samples stored at an a_w of 0.85 and above absorbed moisture in the initial stages of storage and hence shows increase in weight. But later on, due to drip loss, weight loss was noticed in the samples. This is due to the loss of salt and other water soluble constituents in the muscle along with the drip.

The equilibrium moisture contents of the semi-dried salted fillets stored at different a_w are given in Figure 4. At an a_w of 0.75, the semi-dried fillets of all the 3 batches retained moisture content with minimum gain or loss in moisture by way of absorption or desorption. At an a_w of 0.85 and above, the samples absorbed further moisture which resulted in reduction of shelf-life due to accelerated microbial and enzymatic spoilage. It is found that 0.75 a_w is the critical point as far as moisture retention is concerned. This type of behaviour in fish flakes was also noted by Venugopalan and Govindan (1967). Curran and Poulter (1983) also observed similar results in the case of dried salted lisa (*Xenomugil thoburni*).

The physical and olfactory changes noticed in the three batches of shark fillets were more or less similar except for the accelerated rate of spoilage for batch B2 due to its high moisture content. The average score given by the test panel members for the physical and olfactory observations of the samples stored at different constant a_w are given in Table 2. The important physical changes noticed in the fillets during storage at different a_w are yellow discolouration, red discolouration and fungal growth. The period for the first occurrence of yellow/red discolouration and fungal growth in the fillets at different a_w are shown in Figure 5. The samples stored at an a_w of 0.85 and above were found to have yellow discolouration within 14 to 18 days. The visible fungal growth was noticed on samples stored at 0.65 to 0.85 after 26 days of storage. Among the 3 batches, yellow/red discolouration and fungal growth (at higher a_w) were noticed first in batch 2. This may be due to the high initial moisture when compared to other batches which accelerated the enzymatic and microbial spoilage. Red discolouration was noticed in all the batches up to an a_w of 0.43. From Table 2 and Figure 5 we can predict the quality changes and the shelf-life of the fillets under different a_w conditions.

The total bacterial counts of the 3 batches of fillets at the start of the storage study were 5.8 x 10^3, 2.0 x 10^4 and 2.38 x 10^3 for the batches B1, B2 and B3 respectively. The total bacterial counts at the time of equilibrium at different a_w are given in Figure 6. Up to an a_w of 0.65 the bacterial count was low in all the batches and thereafter shot up and reached a maximum count of 6 log at an a_w between 0.90 and 1.00. None of the initial and stored samples had *Faecal Streptococci, E. coli* or Coagulase Positive *Staphylococci*. The total fungi counts when the samples attained equilibrium at different a_w are given in Figure 7. The maximum growth of fungi was between an a_w of 0.50 and 0.85. At a_w of 0.90 and above, no fungus was noticed. Below an a_w of 0.50 its count was negligible. These results are in agreement with the principles on water activity and microbial spoilage (Scott, 1957; Troller, 1983). Scott and Troller have described the growth pattern of bacteria and fungi with respect to a_w. According to them the maximum growth and survival of bacteria occurs between a_w of 0.90 and 1.00. For fungi it is between 0.60 and 0.90. Since the shark fillets were salted to a level of about 20%, the halophilic bacteria might have survived. According to Troller (1983), they have the ability to withstand a_w values of about 0.75.

The sorption data for the semi-dried salted fillets and the calculated a_w's are given in Table 3. The isohalic sorption isotherms for the fillets are given in Figure 8. The isotherms for the three batches of fillets at a_w below 0.75 were very similar but above this value they began to diverge. According to Doe *et al* (1982), a_w of dried salted fish can be expressed as

$$a_w = a_w \cdot a_w n$$

where $a_w n$ is the water activity of the sodium chloride solution present in the fish sample and $a_w o$ is the water activity of the muscle (i.e., salt free, fat free solid fraction). A saturated sodium chloride solution has an a_w of 0.75 and this explains why the isotherms in Figure 8 fall in two parts. A similar pattern is isotherms is also reported by Curran and Poulter (1983) in dried salted lisa. The average monolayer value of salted semi-dried shark fillets was 5.334 gm/100 gm of the solids. The monolayer value can be considered equivalent to the amount of water held absorbed on specific sites (Venugopalan and Govindan, 1967; Karel, 1975). The information of the sorption behaviour and the monolayer value of the fish products are very important in deciding the drying rate and the methods to be adopted for packaging of the product and its storage. They give an indication of the shelf-life of the product under different a_w.

ACKNOWLEDGEMENT

The authors are thankful to Shri M.R. Nair, former Director, Central Institute of Fisheries Technology, Cochin, for granting permission to publish the paper. They are grateful to Dr. P.G. Viswanathan Nair, former Scientist-in-Charge, Research Centre of CIFT, Veraval, for valuable suggestions and encouragements given during the course of the study. They also thank Shri T. Gangadharan and Shri K.U. Shekh of the Research Centre of CIFT for their technical assistance.

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Table 1 Moisure, salt, fat and protein contents of three batches of salted semi-dried shark fillets

Batch	Moisture %	Salt %	Fat % (dry weight basis)	Crude Protein (dry weight basis)
B1	55.97	19.24	0.254	46.20
B2	61.22	19.67	0.330	46.55
B3	54.04	22.83	0.065	44.72

Table 2 Total average score given by five test panel members for the physical and olfactory observations of the three batches (B1, B2 and B3) of semi-dried salted shark fillets stored at different A_w

Batch No.	Days stored	Shelf life under different water activities of								
		0.11	0.32	0.43	0.50	0.65	0.75	0.85	0.90	1.00
B1	3	18	-	20	20	20	20	19	17	-
(20)	7	13	-	16	16	17	19	19	17	-
	14	10	-	14	15	17	19	18	14	-
	22	10	-	13	14	16	15	7	4	-
	32	10	-	12	13	15	15	5	3	-
	48	10	-	11	11	15	9	x	x	-
	55	10	-	11	11	15	x	x	x	-
B2	3	-	17	19	19	19	19	19	-	17
(19)	7	-	14	14	15	17	19	16	-	14
	14	-	12	13	13	17	19	6	-	6
	22	-	11	13	13	16	10	5	-	6
	32	-	11	11	12	15	9	4	-	4
	48	-	11	11	11	11	9	x	-	x
	55	-	11	11	11	9	x	x	-	x
B3	3	-	17	17	18	19	19	19	-	18
(19)	7	-	15	16	16	18	19	15	-	14
	14	-	13	13	15	17	17	15	-	14
	22	-	10	11	13	16	18	12	-	6
	32	-	190	11	11	13	12	8	-	4
	48	-	10	10	11	13	11	x	-	x
	55	-	10	10	10	10	x	x	-	x

- indicates no observation was carried out;

x indicates that the sample was completely spoiled and not taken for observation.

Maximum Total Score = 25; a score of 12 is considered as the lowest tolerance limit for acceptance.

Note: Total score was arrived at by adding up the scores for individual factors. The individual factors considered and their maximum scores allowed are as follows:

Semi-dried appearance:	Excellent-10, very good-9, good-6, satisfactory-4, bad-2, very bad-0.
Colour and odour:	Excellent-5, Very good-4, Good-3, Satisfactory-2, Bad-1, Very bad-0.
Texture:	Semi-dried like-5, marginal loss of semi-dried texture-4, slight loss of semi-dried texture-3, loss of semi-dried texture-2, completely dried-0.

Table 3. Sorption data for salted semi-dried shark fillets and their water activities

Samples	Batch - 1					Batch - 2					Batch - 3				
	M_w	M_s	M_f	M_b	M_w	M_w	M_s	M_f	M_b	M_w	M_w	M_s	M_f	M_b	a_w
Fresh salted semi-dried fillet	0.56	0.19	0.003	0.25	0.77	0.61	0.20	0.003	0.19	0.77	0.54	0.23	0.0003	0.24	0.75
Samples stored in open condition and equilibrated	0.08	0.42	0.002	0.50	0.62	0.20	0.37	0.003	0.42	0.74	0.09	0.44	0.0006	0.47	0.60
Samples stored at different relative humidities of:															
11%	0.54	0.45	0.002	0.47	0.35	-	-	-	-	-	-	-	-	-	-
32%	-	-	-	-	-	0.07	0.36	0.003	0.57	0.44	0.05	0.46	0.0006	0.49	0.35
43%	0.07	0.43	0.002	0.50	0.51	0.08	0.30	0.003	0.62	0.44	0.07	0.44	0.0006	0.49	0.51
50%	0.08	0.48	0.002	0.50	0.56	0.09	0.10	0.003	0.61	0.65	0.09	0.44	0.0006	0.47	0.60
65%	0.14	0.38	0.002	0.48	0.68	0.16	0.27	6.003	0.57	0.69	0.14	0.31	0.0006	0.55	0.67
75%	0.56	0.22	0.001	0.22	0.75	0.59	0.20	0.001	0.21	0.80	0.56	0.20	0.0003	0.24	0.77
85%	0.61	0.16	6.001	0.23	0.82	0.66	0.16	0.001	0.17	0.85	0.59	0.18	0.0003	0.23	0.78
90%	0.64	0.15	0.001	0.20	0.84	-	-	-	-	-	-	-	-	-	-
100%	-	-	-	-	-	0.72	0.12	0.001	0.16	0.88	0.64	0.16	0.0002	0.20	0.84

M_w = Moisture content, M_s = Salt content, M_f = Fat content, M_b = Salt free, fat free, dry mass, a_w = Water activity

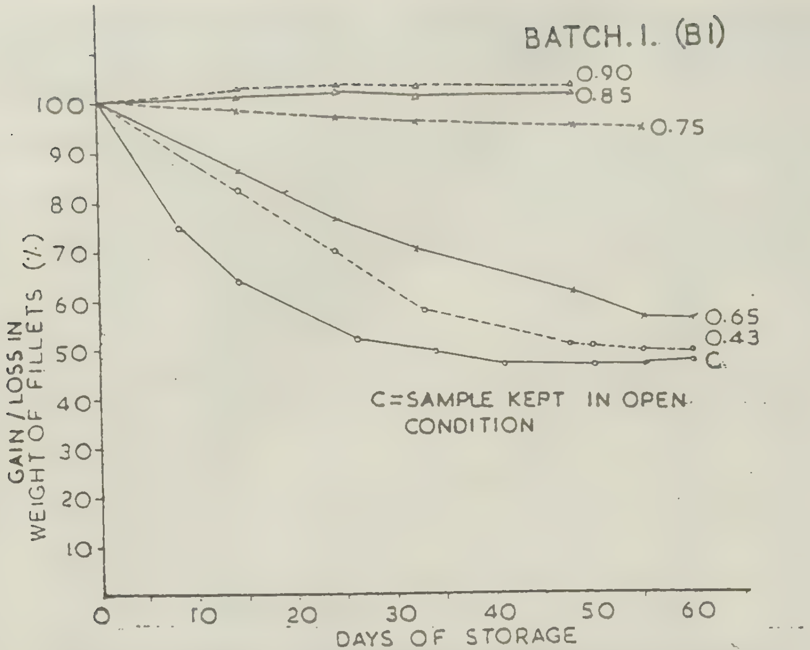

FIG.1. CHANGES IN PERCENTAGE WEIGHT OF SEMI-DRIED SALTED SHARK FILLETS DURING STORAGE AT DIFFERENT (CONSTANT) WATER ACTIVITIES

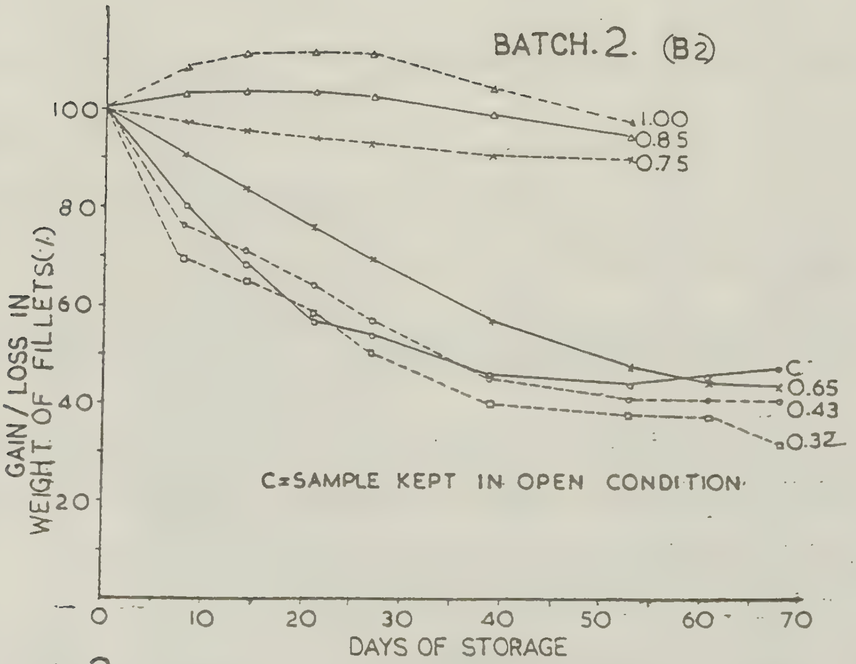

FIG.2. CHANGES IN PERCENTAGE WEIGHT OF SEMI-DRIED SALTED SHARK FILLETS DURING STORAGE AT DIFFERENT (CONSTANT) WATER ACTIVITIES

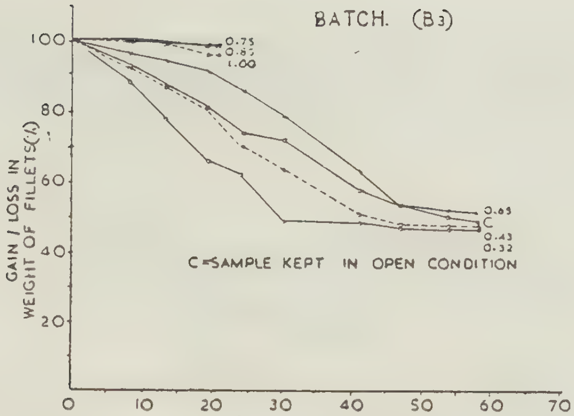

FIG.3. CHANGES IN PERCENTAGE WEIGHT OF SEMI-DRIED SALTED SHARK FILLETS DURING STORAGE AT DIFFERENT CONSTANT WATER ACTIVITIES

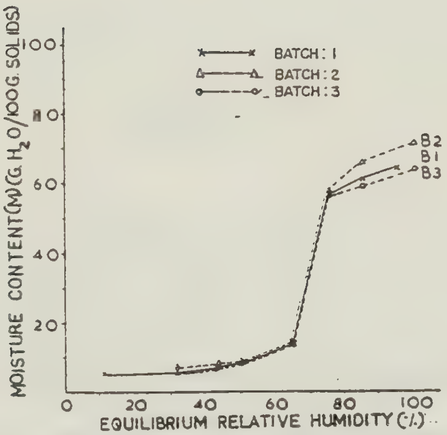

FIG.4 EQUILIBRIUM MOISTURE CONTENT OF SEMI DRIED SALTED SHARK FILLETS (3 BATCHES)

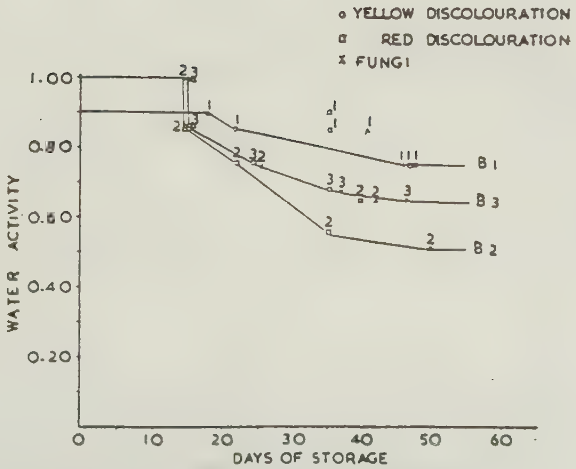

FIG.5. TIME FOR THE FIRST APPEARANCE OF YELLOW / RED DISCOLOURATION AND VISIBLE FUNGAL / COLONIES IN THE THREE BATCHES OF SEMI DRIED SALTED SHARK FILLETS

[1 = BATCH: 1 (B1) 2 = BATCH: 2 (B2) 3 = BATCH: 3 (B3)]

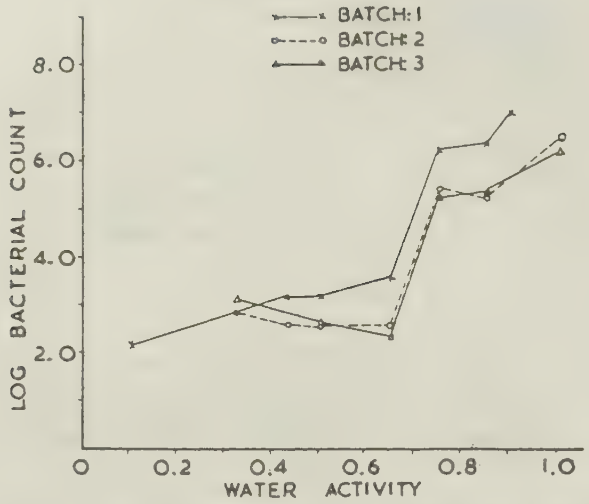

FIG.6. TOTAL VIABLE COUNT OF BACTERIA WHEN THE SEMI DRIED SALTED SHARK FILLETS ATTAINED EQUILIBRIUM AT DIFFERENT (CONSTANT) WATER ACTIVITIES

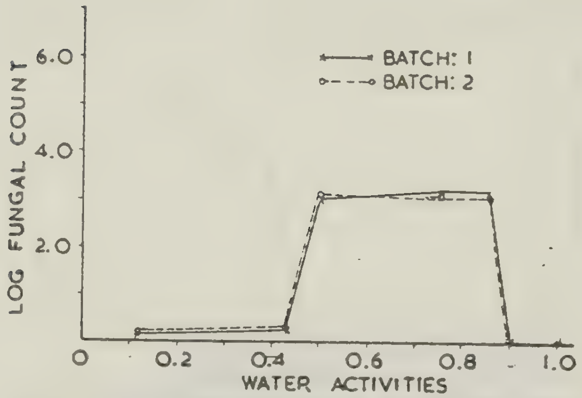

FIG.7. TOTAL COUNT OF FUNGI WHEN THE SEMI-DRIED SALTED SHARK FILLETS ATTAINED EQUILIBRIUM AT DIFFERENT (CONSTANT) WATER ACTIVITIES

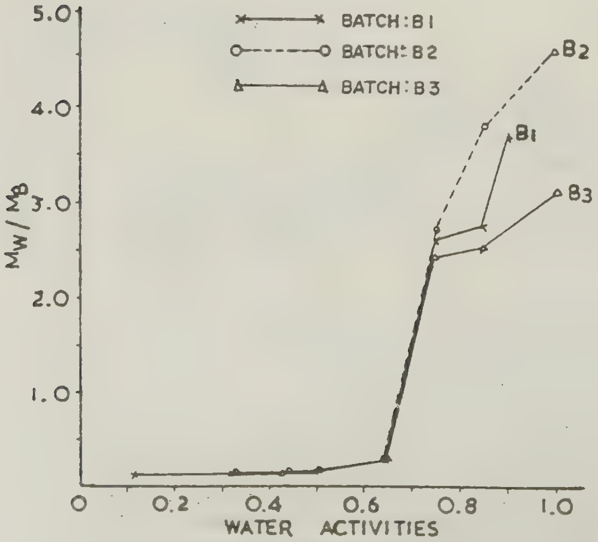

FIG.8. ISOHALIC SORPTION ISOTHERMS OF SEMI-DRIED SALTED SHARK FILLETS

MEASUREMENT OF WATER ACTIVITY AS AN INDEX OF OPTIMISING SALT CONCENTRATION IN BRINE USED FOR THE WET CURING OF MACKEREL (*Rastrelliger kanagurta*)

by

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ABSTRACT

Wet salting with saturated brine is widely used in traditional processing. This paper makes an attempt to find the optimum salt concentration which is required to be maintained in brine used for wet curing of mackerel (*Rastrelliger kanagurta*) prior to drying. Isohalic sorption isotherms for different dried products of mackerel when pre-treated with different concentrations of brine are established. The brine concentration of 11% for wet salting of Indian mackerel is recommended for making a dried product having 35% moisture content (on wet basis) giving an a_w of 0.75.

INTRODUCTION

Salting and subsequent sun drying have been traditional methods for long term preservation of fish for centuries. Although dry salting is commonly practised, wet salting with saturated brine is also widely used in traditional processing. Due to rising cost of salt, the processors often use the saturated brine repeatedly which at times results in poor quality dried products with very poor shelf lives. Rubbi *et al,* (1983) recommended the use of a 10% brine for production of solar tent dried pomfret.

In the present investigation, attempts were made to find whether saturated brine is essential for wet salting of fish and also to find the optimum salt concentration to produce a quality product. Water activity measurement and its relation with moisture content of dried fish are used to limit the requirement of salt.

Water activity is a measure of "free" or loosely bound water within a food stuff. Scott (1936) developed the concept of water activity and later its application was extended to dry fish production and quality assessment by several workers (Poulter *et al,* 1982; Doe *et al,* 1982, 1983, and Doe, 1985).

An understanding of the sorption behaviour of fish is very important in determining the amount of drying needed to produce a dried fish with a given shelf-life. As moisture is gradually removed during drying, water activity decreases progressively along with sorption isotherm. When the fish is dried properly the desired water activity, needed to prevent spoilage, should be attained. This is the most important criteria in determining the shelf life of a dried fish.

Water activity measurement of dried fish was reported to be a good criteria for predicting the shelf life of fish (Poulter *et al,* 1983, Doe, 1985).

MATERIALS AND METHODS

Fresh mackerel (*Rastrelliger kanagurta*) were purchased from local fish markets and were split dorsally, cleaned and washed well with potable water. The entire quantity was divided in six lots for carrying out wet salting in six different brine concentrations namely, 5%, 7%, 10%, 11%, 18% and 26% . The wet salting was carried out at ambient temperature 24 ± 3°C for 6 h, 18 h and 24 h. The quality of the wet cured fish was assessed by determining the total volatile basic nitrogen (TVBN).

The fish were cured for 24 h and dried in a mechanical dryer for 18 h at 45-55°C, 65% R.H.

The moisture, salt and fat contents of the fish muscles at different intervals during the course of drying were determined by the methods of AOAC (1980). The total volatile basic nitrogen (TVBN) was determined by the micro diffusion method of Conway (1947) from the trichloroacetic acid extract of the fish muscle.

The experimental data on moisture, fat and salt contents and the water activity (a_w) to determine the isohalic sorption isotherm.

RESULTS

Table 1 gives the change in total volatile basic nitrogen of the fish muscle during the wet-salting of mackerel in six different brine concentrations. The results revealed that there were no appreciable changes in the values of TVBN for fish samples salted in brine solutions of concentration from 11 to 26%. However, on salting at 10% brine, TVBN values increased to 21.7 mg%, 46.4 mg% and 94.6 mg% after 6 h, 18 h and 24 h respectively. More pronounced increase in TVBN values were noticed when salted in low concentrations of brine namely 5 and 7 percent. Further, it was noticed that excessive fermentation took place after 10 h when the fish samples were kept in 5 and 7 percent brine.

Figure 1 shows the isohalic sorption isotherms obtained by plotting M_w against a_w at different stages of drying for all the six different concentrations of brine that were used during wet-salting. It was observed that the fish salted in 11, 28 and 26 percent brine gave the same isohalic sorption isotherms. At 35% moisture level of dry fish on wet basis (M_w = 0.538) which is desired by processors in India, the water activity of the dried fish worked out to be 0.75 for all the fish which were salted in 11, 18 and 16 percent brine. The fish which were brined at 5, 7 and 10 percent brine solutions were found to have different isotherms as seen in Fig. 2 and the water activities were 0.88, 0.84 and 0.81 respectively for dried products at 35% moisture level.

CONCLUSION

The study reveals that to obtain a desired water activity (a_w) of 0.75 in the dried product with 35% moisture content on a wet basis, wet salting for 24 h at a brine concentration of not less than 11 percent is required. However, to achieve an a_w of 0.75 in dried products, the moisture content on wet basis of the products should be 18%, 23% and 24.24% respectively if the wet salting is done with 4, 7 and 10 percent brine. This will no doubt increase the cost of drying. Moreover, the dried products were found not to be of good quality compared to those salted in 11 percent brine or higher concentrations.

The treatment with high concentrations of brine to the extent of 26% NaC1 does not give any added advantage compared to the fish treated with 11 percent brine. Rather fish treated with higher concentrations of salt lead to dried products with low shelf lives as a result of high rancidity.

Repeated wet salting in the same brine liquor may be carried out provided the brine concentration does not fall below 11%.

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Storage of Solar-Tent Dried Pomfret (*Stomateus spp.*) Inc: "The Production and Storage of Dried Fish", D. James (Ed.) FAO, Fish. Report 279: 93-100.

Table 1 Changes in total volatile basic nitrogen content (TVBN) of cured mackerel on wet curing at 24 ± 3°C

Concentration of Brine %(w/v)	TVBN mg/100g (Period of wet curing)			Occurrence of fermentation after 10 h. of wet curing
	6h	18h	24h	
5	52.6	106.4	169.3	+ + +
7	37.8	83.8	142.7	+ + +
10	21.7	46.4	94.6	+
11	4.4	9.7	24.3	Nil
18	2.6	5.1	7.1	Nil
26	1.2	1.4	1.6	Nil

The values of TVBN given in the table represent average of three readings. The notations - (+) means slight fermentation and (+ + +) means excessive fermentation.

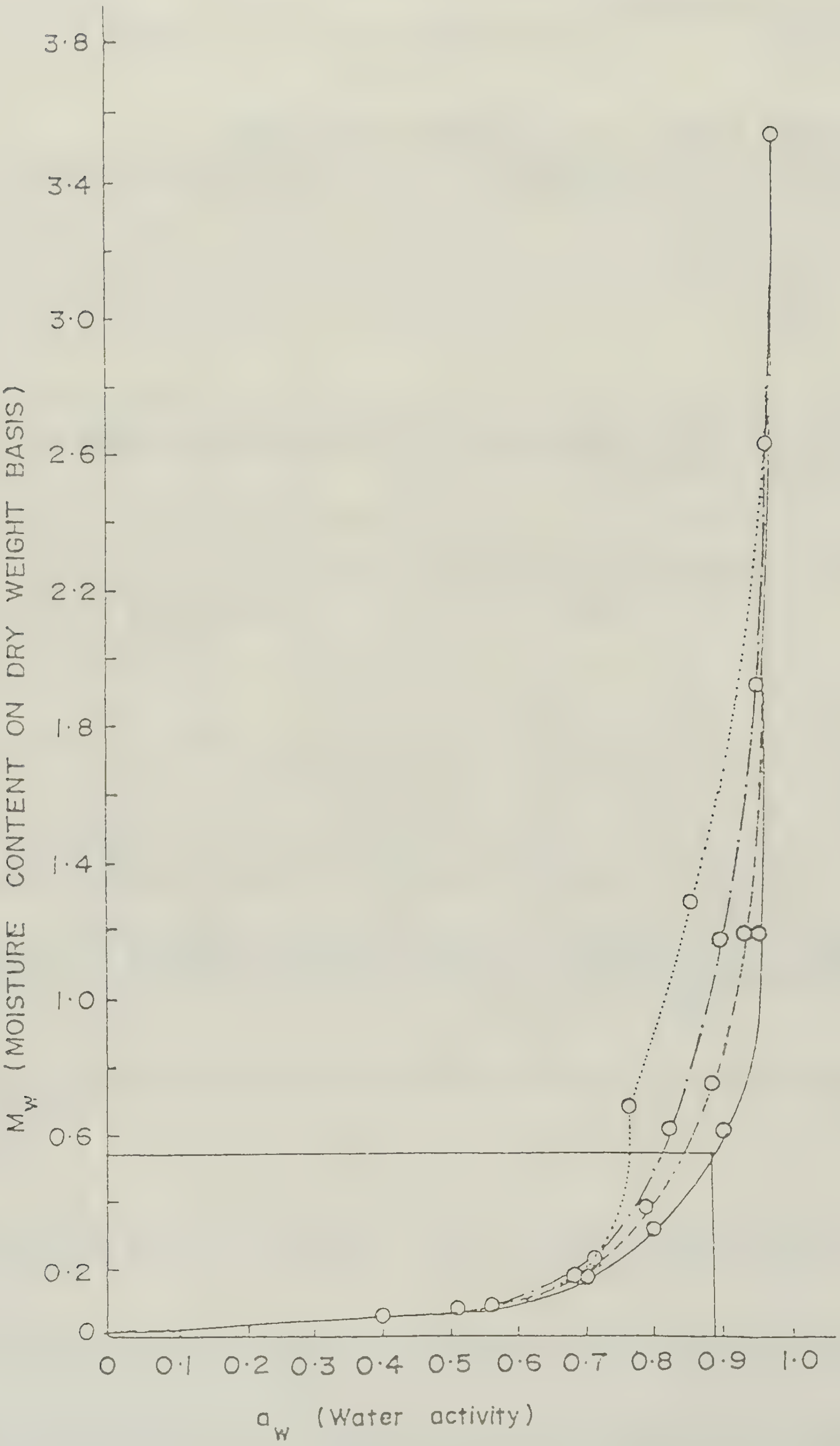

Fig.1. ISOHALIC SORPTION ISOTHERMS SHOWING M_W AGAINST a_W

(o———o 5% , o- - - -o 7% , o—·—o 10% , o·······o 11% & above)

THE EFFECT OF DIFFERENT SALTING PROCEDURES AND QUALITIES OF RAW MATERIAL ON SOME NUTRIENTS DURING PROCESSING OF SALTED-DRIED MACKEREL

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ABSTRACT

Salting and drying is one of the most important methods of processing fish and is predicted to continue to be nutritionally and economically important in the foreseeable future. The study investigated the effect of different methods of salting and the different quality of raw material on the quality of fish during processing and one months storage using mackerel *(Scomber scombrus)*. There were no significant changes in proximate composition in mackerel during processing and storage when expressed on a dry weight basis salt free. However, considerable changes in thiamine, riboflavin and available lysine occurred during salting, drying and one months storage, which losses of between 26 and 49% of thiamine, 16 and 35% of riboflavin and 16 and 38% of available lysine were incurred. The pickle salting method resulted in the lowest losses of all three of these nutrients whilst the greatest losses were using 15% brine salting method. The effect of quality of raw material on the quality of end product of salted-dried mackerel show that better quality raw material fish produced a better nutritional quality with reference to available lysine, thiamine and riboflavin content of the end product.

INTRODUCTION

In some countries including Indonesia, fish is one of the important sources of low-cost dietary protein and salted-dried fish is part of the staple diet. However, little research has been reported on the nutritional quality changes during processing and storage of this product. Fish oils are very susceptible to atmospheric oxidation, and in salted-dried fish they have little protection from this process. Salt and haemoprotein may also catalyse this oxidation, ultimately giving products which can undergo the non-enzymic browing reaction, which reduces the nutritional value of the protein by reaction with essential amino acids such as available lysine. The extensive lipid oxidation could also decrease levels of vitamin, such as thiamine and riboflavin, and these changes in nutritional quality during processing and storage have also been determined in this study.

MATERIALS AND METHODS

Materials

Fresh mackerel *(Scomber scombrus)* were purchased from Grimsby Fish Dock. The weight of mackerel ranged from 359 to 512 gram. Salt of food grade was obtained from J.W. Moores Ltd., Grimsby. All reagents used were of analytical reagent grade, unless otherwise specifically mentioned.

Processing

The mackerel were processed to form salted-dried mackerel by the following stages; dressing, washing, salting, drying and one months storage. Four methods of salting were applied, namely 15% brine, saturated, pickle and dry salting.

The effect of the raw material on the quality of the salted-dried mackerel was investigated using two different qualities of mackerel as starting material. Two qualities of fresh mackerel were obtained by dividing the fresh mackerel into batches and treating as follows :

a)	Batch A:	Fresh mackerel (grade 8 of hedonic scale system SPI-KAN, 1982) immediately processed.
b)	Batch B (aged):	The fresh mackerel were kept at chilling temperature for 75 hours prior to processing. This produced mackerel of grade 7 (hedonic scale).

The chemical composition of the samples, from each treatment were analysed before salting, after salting, after drying and after one months storage.

Analytical techniques

Proximate analysis

Protein was determined by the Kjeldahl method, fat by Bligh and Dyer procedure, moisture by drying method (Egan *et al,* 1981) and salt by titration (FAO, 1981).

Available lysine

The available lysine determination by HPLC was by the method of Carpenter (1960) modified by Peterson and Warthesen (1979).

Thiamin (vitamin B1)

The method used was the thiochrome-fluorometric method based on the AOAC method (1984) modified by Superno (1988), and developed by Surono (1991).

Riboflavin (vitamin B2)

The method used was the fluorometric method based on the AOAC method (1984) and Osborne and Voogt's method (1978) modified by Surono (1991).

RESULTS AND DISCUSSIONS

Proximate Composition of the salted-dried fish

There were no significant changes in proximate composition during salting, drying and 30 days of storage when expressed on a dry salt free basis. The apparent change in composition on a wet basis were due to some loss of moisture and uptake of salt which result in the changes in water activity. This agrees with results of Maruf *et al,* 1990. However, salting, drying and storage had a considerable effect on the nutritional quality of the products.

Available lysine changes

The changes in available lysine due to the different quality of raw materials and different methods of salting during processing and storage are presented in Figures 1. It can be seen that processing and storage considerably reduced the available lysine content in salted-dried mackerel. This agrees with the results of the previous work (Surono, 1991). Generally, the greatest losses were incurred during the drying stage. This could be due to the air flow (at 35-40°C) containing oxygen which was employed during this stage, causing an acceleration in lipid oxidation, since oxygen is involved in the propagation stage of lipid oxidation. Therefore, the higher oxygen pressure the higher rate of oxidation (Labuza, 1971). This could in turn lead to the destruction of available lysine. Increases in PV were observed at this stage in previous experiments (Surono, 1991).

Differences in quality of raw material and in methods of salting affected the changes of available lysine during processing and storage of salted-dried mackerel. Figure 1 demonstrates that, in general, the value of available lysine of fresher samples (A) decreased less than the aged (B) samples. As shown in Figure 1, the initial values of available lysine of batch A and B were similar, but subsequent values were much lower in aged samples (B). The results for unprocessed mackerel (30 and 29 mg/g dwbsf) agree with the previous work (Surono, 1991).

The different methods of salting affected the level of available lysine. As shown in Figure 1, the MD samples in both batches gave the lowest value of available lysine. This could have occurred due to the fact that the lipid oxidation may have been greater than in the other methods of salting, as was observed in the previous experiments (Surono,1991). The MP samples (in both batches), on the other hand, gave the highest values of available lysine after salting, which were 30 and 28 mg/g dwbsf respectively.

The drying stage resulted in the greatest losses in available lysine. The highest losses were observed in M15B samples (in both batches), which may be expected as M15B had the highest water activity (0.8), in which greatest deterioration (microbial and chemical), would be expected to occur. The MP samples, on the other hand, had the highest available lysine contents.

During storage, further decreases in available lysine occurred. This was also found in previous experiments. After one months storage, the lowest losses of available lysine occurred in the MP samples of both batches. In the fresher batch (A), the total losses of available lysine in MP samples were 16 percent, followed by MD (19), MSB (19), and M15B (33). Thus the use of better quality fish and pickle curing is recommended to minimise losses in available lysine.

Changes in thiamine

The initial value of thiamine in fresh mackerel was 0.21 mg/100g. This is slightly higher than previous reports of 0.17 - 0.20 mg/lOOg (Tarr, 1962; Nettleton, 1985), but this could be due to the variation in samples. The changes in thiamine content using different qualities of raw material and salting methods, and with drying and one months storage are presented in Figure 2.

Figure 2 show that the lower quality of raw material suffered more losses in thiamine during processing and storage of salted-dried mackerel. The 75 hours aging had no noticeable effect on the initial values for thiamine content. However, during salting, the aged batch (B) lost more thiamine than the fresher batch. This might be due to leaching of compounds during salting, since the lower quality samples (batch B) lost more moisture, in which thiamine would be soluble, than the fresher samples. With the aged batch, the M15B and MSB samples gave lower thiamine values than MD and MP samples (Figure 2A). During drying, the thiamine content of the aged batch (B) decreased much more than that of the fresher batch (A), with 29-38 percent and 6-20 percent decreases respectively.

During one months storage, the destruction of thiamine still occurred in both batches but at a slower rate with little loss in the aged batch. After one months storage, the overall losses of thiamine in the fresher batch were lowest in the MP samples (26%) followed by MD (28%), MSB (32%) and M15B (37%). The same sequence was observed in the aged batch but with losses of 33, 42, 46 and 49 percent respectively. These changes may be due to microbiological, autolytic, and lipid oxidation reactions which could accelerate the destruction of thiamine. Mackelsen and Makdani (1975) found that breakdown of thiamine could be due to bacteria producing thiaminase. Moreover, Dwivedi and Arnold (1973) reported that thiaminase may be present originally in mackerel. Another factor is that salt (NaCl) is a univalent electrolyte, which promotes the degradation of thiamine (Fernandez *et al,* 1986).

From these results of the thiamine losses, it can be recommended that the use of better quality raw material and the pickle salting method are the best way to reduce the losses of thiamine in salted-dried mackerel.

Changes in Riboflavin.

The effect of different qualities of raw material, different methods of salting, followed by drying, and storage of salted-dried mackerel are presented in Figure 3.

In general, the fresher batch (A) gave lower losses of riboflavin than the aged batch during processing and storage. The initial value of riboflavin in both batches were similar at 0.140 and 0.138 mg/lOOg wwb (wet weight basis) or 0.37 mg/lOOg dwbsf (dry weight basis salt free).

During salting the effect of different methods of salting showed that in the fresher batch, MP samples gave the lowest losses of riboflavin followed by MSB, MD and M15B with values of 0.37, 0.36, 0.36, and 0.35 mg/lOOg dwbsf respectively. A similar sequence, but with slightly higher losses, was recorded in the aged batch.

As with thiamine, the drying period was the stage during which most losses of riboflavin occurred. The lowest losses in batch A occurred in MP samples followed by MD, MSB and M15B, giving 9, 12, 16, and 19 percent losses respectively from the initial value. Similarly, the same sequence was recorded in the batch B but with higher losses, namely 11, 17, 22, and 28 percent respectively. Generally, the higher losses reflected the higher water activity, which would be expected to correlate with a higher microbial activity, but additional factors e.g. interreaction of riboflavin with lipid oxidation products may also have an effect.

During the one months storage, degradation of riboflavin still occurred although it was slower. This could reflect the decreasing water activity of all of the samples, in which bacteria could still grow. After one month's storage, the overall riboflavin losses in batch A of MP, MD, and M15B were 16, 17, 22, and 27 percent respectively. The same sequence was observed in the aged batch but with higher losses of riboflavin.

Again, these results demonstrate that the use of the pickle salting method and better quality of raw material of mackerel are the best way to reduce the riboflavin losses during processing and storage of salted-dried mackerel.

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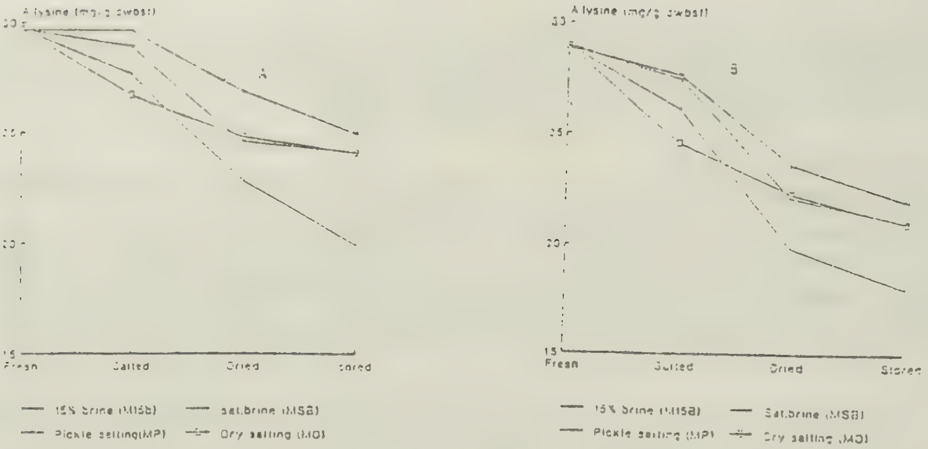

Figure 1. The changes of Lysine of A samples (fresh) and B samples (aged) during processing and one months storage of salted-dried mackerel

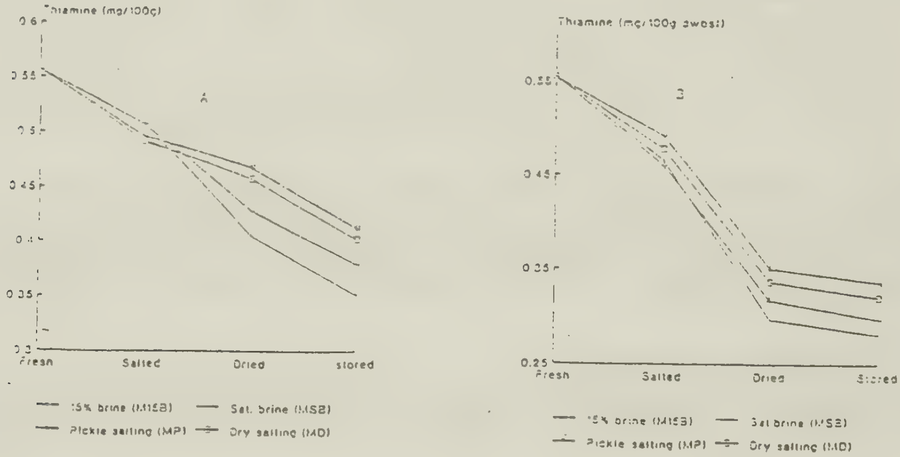

Figure 2. The changes of Thaiamine of A samples (fresh) and B samples (aged) during processing and one month storage of salted-dried mackerel

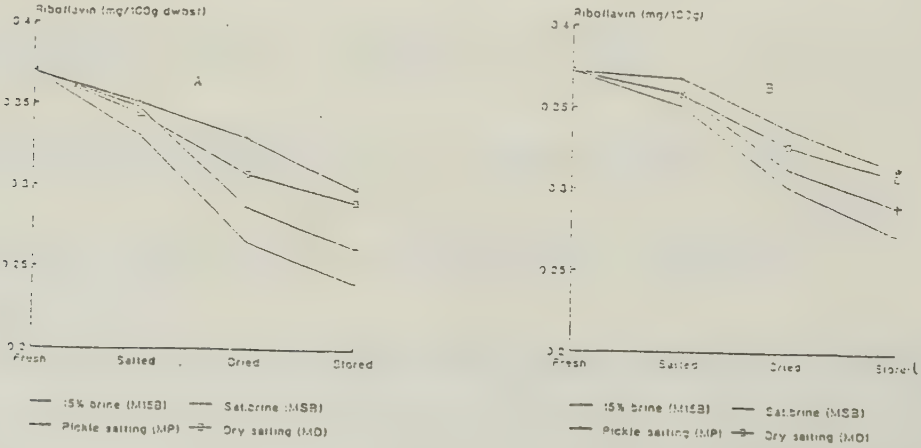

Figure 3. The changes of Riboflavin of A samples (fresh and B samples (aged) during processing and one months storage of salted-dried mackerel

USE OF DRYACIDE, DELTAMETHRIN AND CYCLOPROTHRIN TO CONTROL INSECT INFESTATION ON DRIED SALTED FISH

by

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ABSTRACT

Insect attack on dried fish, particularly by dermestid beetles, causes significant losses in Indonesia and measures that have been taken to overcome the problem have met with little success. Many insecticides are available in the markets but little work has been done to investigate the range of material employed and their effectiveness to reduce insect infestation on dried fish products.

This paper reports the effect of treatment of packaging materials with chemical materials on subsequent levels of dermestid infestation of bags of dried fish. It was found that the desiccant Dryacide when sprinkled on the inner surfaces of dried fish packaging failed to protect fish loss due to dermestid infestation. Deltamethrin at 0.0025%, a.i., sprayed on the outer surface of dried fish packaging effectively protected fish from dermestid infestation only for two weeks. Higher concentrations gave longer protection.

Deltamethrin sprayed at 0.05mg/m^2 on the outer ply of packing material protected unsalted dried anchovy from insect penetration for more than three months, while cycloprothrin (0.5mg/m^2) gave protection for two months.

INTRODUCTION

The fisheries industry is a significant part of Indonesia's economy and the government endeavours to increase fishery production through better resource management, aquaculture, research, training, extension, and the implementation of appropriate post-harvest technology. Over the last ten years losses of fishery products in Indonesia have been reduced through transfer of technology, a better perception of fish quality, and the increased economic importance of fisheries in both domestic and world markets.

Ongoing fisheries projects and collaborative research include methods of handling and preserving fresh fish, processing and quality control, all combined with marketing and socio-economic studies. Besides modern processing for export and domestic products, these research programmes are also examining traditional processing of seafood, mostly cured fish products, with a major goal in the optimization of these traditional methods to reduce post-harvest losses. Surveys and experimental work have identified that insect infestation causes significant losses to traditional fish products. Hanson and Esser (1985) indicated that blowflies caused considerable deterioration of large or thick-fleshed fish during drying. Later, during storage, insects, e.g., *Dermestes, Necrobia* and *Piophila*, and mites, are responsible for damage on dried fish (Indriati *et al.*, 1985). The extent of these losses due to insects has been reported by Indriati *et al* (in this proceeding).

Measures taken to reduce insect infestation include the use of insecticides on wet fish and storage at low temperatures, proper packaging and/or regular cleaning and repackaging for dried fish. The application of insecticides to protect drying fish from blowfly infestation has been reported by several investigators (Walker and Donegan, 1984; Hanson and Esser, 1985; Esser *et al.*, 1986). Insecticides are also applied to protect dried fish from dermestid beetle infestation during storage (Taylor and Evans, 1982; Duguet *et al.*, 1985; Esser *et al.*, 1986; Golob *et al.*, 1987). Insecticides employed include pirimiphos-methyl, chlorpiriphos-methyl, tetrachlorvinphos and pyrethrum synergised with piperonyl butoxide, deltamethrin, and metacriphos. Results suggest that deltamethrin and pirimiphos-methyl have potential for use as protectants of dried fish. However, treatment of fish with insecticides poses a great danger to human health, especially when the user is ill-informed about the toxicity of the insecticides he deals with. Therefore this research was conducted to seek safer methods, both physical or chemical, that will prevent insect penetration of bags.

MATERIALS AND METHODS:

1. Dried fish:

Dried anchovy (*Stolephorus spp.*) were bought from a dried fish warehouse at Kalibaru. Fish were kept in a drying oven at 50°C for an hour to kill any insect eggs, larvae or adults. The fish were then sieved to remove broken fish, frass and dust, and the collected fish was divided into three equal batches. The first batch was packed in bags treated with Dryacide, the second batch was in bags treated with deltamethrin, and the third batch was packed in untreated bags as control.

2. Packaging:

First trial

Fifteen triple-walled bags of 30 x 18 x 12 cm were made from a cement paper bag which was placed over another bag to make a triple-walled bag. The surface of the outer bags was sprayed with deltamethrin (3 bags), and the inner surface of other three bags was sprinkled with Dryacide. Unsprayed bags (9 bags) served as controls.

Insect challenge:

Dryacide:

Eleven adults and 25 larvae of dermestid were packed with 700 g fish in each of dryacide treated and untreated bags. These bags were kept in insect proof containers (60 x 45 x 45 cm) to prevent insect infestation from outside the bags.

Deltamethrin:

Each of two separate containers were filled with 25 dermestid adults and 73 larvae. Next, untreated bags and bags treated with deltamethrin containing 700 g fish each were placed into the appropriate container and challenged by the dermestids.

Second trial

Thirty-six triple-walled bags of 30 x 18 x 12 cm were made from a cement paper bag which was placed over another bag to make a triple-walled bag. The surface of the outer bags was sprayed with deltamethrin (9 bags), and the inner surface of other nine bags was sprayed with Dryacide. Unsprayed bags (18 bags) served as controls.

Insect challenge:

Dryacide:

Insect population consisted of a pair of *Dermestes carnivorous*, a pair of *Dermestes maculatus*, and 10 dermestid 5th instar larvae were added to 250 g fish in each of the Dryacide treated bags and the untreated bags. These bags were kept in a closed container to prevent insect infestation from outside of bags.

Deltamethrin:

To each of two separate containers (60 x 45 x 45 cm) were placed ten pairs of *Dermestes maculatus*, and 75 dermestid 5th instar larvae. Next, untreated bags and bags treated with deltamethrin containing 700 g fish were placed into the appropriate container and challenged by the dermestids.

3. Insecticides:

a. Dosage:

Deltamethrin was diluted in water (1ml/l) - 0.0025%
Dryacide was put in a shaker that will pour out about 0.5 g per bag.

b. Application:

Deltamethrin was sprayed evenly on the outer surface of the bags (± 5 ml/bag).
Dryacide was sprinkled evenly on the inner surface of the bags (± 0.5 g/bag).

Third trial

1. Dried fish:

Dried anchovy (*Stolephorus spp.*) were bought from a dried fish warehouse at Kalibaru. Fish were kept in a microwave oven at high heat for 60 seconds to kill any insect eggs, larvae or adult, then were sieved to remove broken fish, frass and dust. Fish were divided into three batches. The first batch was packed in bags treated with deltamethrin, the second was in bags treated with cycloprothrin, and the third in untreated bags as control.

2. Packaging:

Thirty-six triple-walled bags of 30 x 18 x 12 cm were made from a cement paper bag which was placed over another bag to make a triple-walled bag. The surface of the outer bags was painted with deltamethrin at 0.05 mg/m^2 (12 bags) and cycloprothrin at 0.5 mg/m^2 (12 bags). Unsprayed bags (12 bags) served as controls.

Insect challenge:

Each of six separate containers was filled with *Dermestes carnivorus* (10♀ + 10♀), *D. maculatus* (20♀ + 20♂). Then, untreated and treated bags containing 250 g fish were placed into the appropriate container and challenged to those dermestids. After a week many adult dermestids were found dead, and the population was replaced by *Dermestes carnivorus* (11♀ + 11♂), *D. maculatus* (5♀ + 6♂).

3. Insecticides:

a. Preparation

Tapioca starch solution was made by mixing 50 g tapioca starch in 2 l hot water. When cool, tapioca starch was thoroughly mixed with the appropriate insecticides. A volume of 1.8 ml of either deltamethrin or cycloprothrin was mixed into 300 ml starch solution.

b. Application

Out of these solutions 30 ml was applied on outer surface of paper bags on an area of 0.09/m^2, so that deposits on the paper would be 0.05mg/m^2 for deltamethrin and 0.5mg/m^2 for cycloprothrin. The insecticide-coated bags were allowed to dry for 24 hours before being used in packaging the anchovies.

4. Location:

Treated and control bags containing dried anchovy were stored in MRFS laboratory, 27-30°C, 68-72% RH.

5. Sampling:

Bags containing dried anchovies were sampled on or about days 7, 30, 60 and 90. At each sampling, bags were emptied of their contents for observations on:

- physical damage made by insect on packaging (number of holes)
- weight loss of dried anchovy
- insect population on dried fish (number, life-stages)
- other infestation (mould, bacteria).

6. Data analyses

Data were statistically analyzed by a one-way ANOVA at $\alpha = 0.05$.

RESULTS AND DISCUSSION:

Dryacide, a sorptive silica dust, can be expected to exhibit insecticidal activity to control insect infestation on dried fish. Desmarchelier and Dines (1987) showed the effectiveness of this silica containing material to kill insects in stored wheat. However, this study indicates that dryacide did not provide any protection to the anchovy inside the multi-walled paper bags (Figure 1; Tables 1 and 2).

While Dryacide can be expected to exhibit a broad spectrum of insecticidal activity (Agnew Clugh Limited, unpublished; Desmarchelier and Dines, 1987), tests confirmed that the toxicity of Dryacide to grain insects was inversely proportional to relative humidity (Patourel, 1986). In this experiment the relative humidity inside the bags was more than 70%. Another probable reason is that high moisture content of the dry anchovy (14.4-15.5%) alleviated the toxicity of Dryacide to dermestid populations. Patourel (1986) tested the toxicity of similar sorptive silica dusts in samples of whole wheat to adult population of four species of grain beetles and found that the tolerance of those species to the dust treatments increased with increasing moisture content of wheat (greater than 12%). Furthermore, in this trial Dryacide was sprinkled on the inner surface of the bags and was not mixed with the fish, thus the probability of contact with the dermestids was very small. This suggests that Dryacide is not appropriate for controlling dried fish insects, considering that the lowest moisture content of commercial dried fish was around 13% and that fish are usually kept at high humidity (> 60%).

Golob *et al.* (1987) showed that tilapia species dipped for 4 seconds in 0.002% i.e., deltamethrin solution could be effectively protected from dermestid infestations for up to six months. A similar result was obtained by Duguet *et al.* (1985). However, application of these pyrethroid compounds on human food is very risky. Deltamethrin sprayed at 0.0025% on the outer surface of the bags protected the fish in bags from dermestid infestation, but only for two weeks, beyond which it was ineffective (Tables 3 and 4). Dermestids that were ready to pupate penetrated the bags, especially the untreated bags (Figure 2), and no mortality of pupae was recorded. This suggests that a higher concentration of deltamethrin is required to give a longer protection.

In the third trial deltamethrin and cycloprothrin were applied as slurry (mixed with tapioca solution) on the bags with a concentration of 0.05mg/m^2, respectively. The new insecticide, Cycloprothrin, invented by the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia, has shown insecticidal activity against various pests in paddy rice, vegetables, top fruits, citrus, tea pastures, etc. It also has very low toxicity to mammals and humans (Nippon Kayaku, 1982).

Table 5 shows that deltamethrin sprayed at 0.05mg/m^2 protected unsalted dried anchovy from insect penetration for more than three months and Cycloprothrin (0.5g/m^2) gave protection for only two months. Deltamethrin had a stronger repellant effect on dermestids causing a high mortality of the dermestid population outside the bags. Multi-walled cement paper bags did not cause condensation on product, but could not totally prevent the content from dermestid attack.

CONCLUSION:

1. Dryacide sprinkled on inner surface of dried fish packaging does not effectively protect fish from dermestid infestation.

2. Deltamethrin at 0.0025%, i.e., sprayed on outer surface of dried fish packaging, protected the fish effectively from dermestid infestation for only two weeks.

3. Deltamethrin sprayed at $0.05g/m^2$ protected unsalted dried anchovy from insect penetration for more than three months.

4. Cycloprothrin ($0.5g/m^2$) gave protection only for two months.

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Table 1. Number of the dermestids remaining alive after exposure to dryacide within bags of fish initially containing 4 adults and 10 big larvae of dermestids and 250 g dried anchovies

Treatment	Number of insects after storage		
	One week	Two weeks	Three weeks
	Average (n = 3)		
Dryacide	24.67	32.33	86.33
Control	21.67	41.00	102.33

Table 2. Analysis of variance (log(x + 1) transformed data) on the number of larvae in dryacide-treated and untreated bags containing 250 g dried anchovies

Source	df	SS	MS	F	P value
Between treatments	1	0.001	0.001	0.006	0.941
Error	16	1.985	0.124		
Total	17	1.986			

Table 3. Number of deltamethrin-treated and untreated bags invaded and the number of insects in the bags containing 250 g dried anchovies

Treatment	Number of bags invaded and the number of insects after storage					
	One week		Two weeks		Three weeks	
	Invaded bags	Insects	Invaded bags	Insects	Invaded bags	Insects
	(n = 3)					
Deltamethrin	1/3	0	0/3	0	1/3	21
Control	1/3	0	3/3	19	1/3	38

Table 4. Analysis of variance (log(x + 1) transformed data) on the number of holes on deltamethrin-treated and untreated bags containing 250 g dried anchovies

Source	df	SS	MS	F	P value
Between treatments	1	0.258	0.258	2.35	0.145
Error	16	1.755	0.110		
Total	17	2.013			

Table 5. Invasion of deltamethrin and cycloprothrin treated and untreated bags of anchovies with time after presentation to a dermestid population and the number of insects in the bags

Treatment	Number of bags invaded and the number of insects after storage					
	30 days		60 days		90 days	
	Invaded bags	Insects	Invaded bags	Insects	Invaded bags	Insects
	(n = 4)					
Deltamethrin	0/4	0	0/4	0	0/4	0
Cycloprothrin	0/4	0	2/4	31	2/4	18
Control	4/4	91	3/4	17	4/4	181

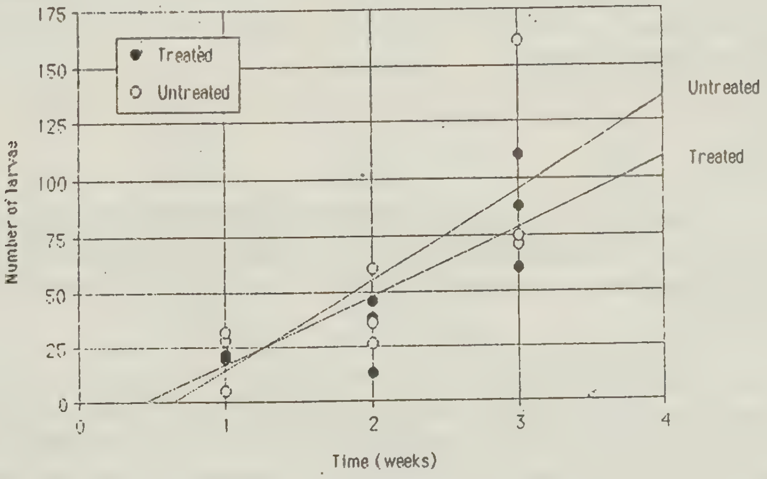

Figure 1. Number of larvae in dryacide-treated and untreated bags containing 250g dried anchovies, 4 adults and 10 larvae of dermestids.

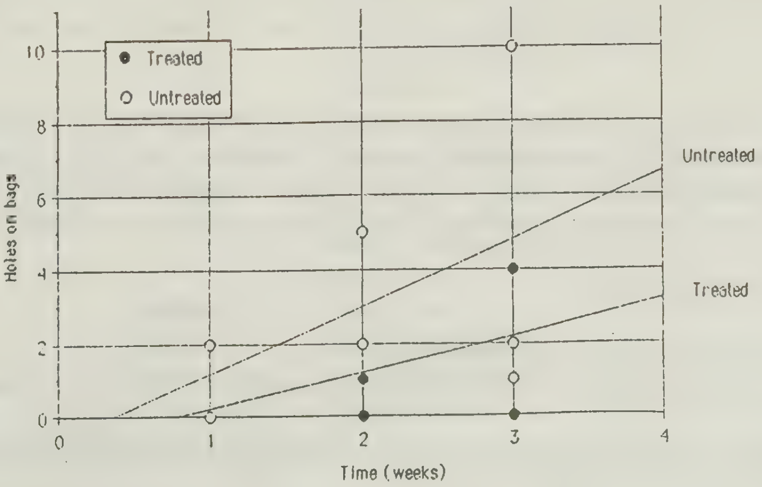

Figure 2. Number of holes penetrated by dermestids on deltamethrin-treated and untreated bags containing 250g dried anchovies

INSECT INFESTATION OF DRIED FISH DURING DISTRIBUTION AND MARKETING

by

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ABSTRACT

Considerable losses occur during distribution and marketing of dried fish due to insect infestation. *Chrysomyia, Piophila, Dermestes* and *Necrobia* species and mites are the most frequent pests associated with the infestations. Levels of infestation vary significantly depending on the stage of distribution in the marketing chain. Infestation levels of 30 percent have been recorded during processing, 5 percent at wholesalers and 60 percent and 35 percent at large and small retail outlets respectively. These levels were closely related with the time of storage at each stage of marketing chain.

The vulnerability of dried fish to insect infestation resulted in the introduction of various control measures which result in additional cost to processors and distributors. Reprocessing and repackaging are among the most common practices employed to counter this problem. Storage of dried fish in a chilled room, a most suitable method was only utilized by some large retailers. In a few cases, non-recommended insecticides were used by some processors to control insect infestation during the drying of certain large fish species.

INTRODUCTION

As a tropical country, Indonesia has stable and optimal conditions of temperature and humidity for the growth of insects all year round. The presence of pest insect species causes problems in the distribution and marketing of dried fish. Traditionally, processed - such as sun dried, smoked and fermented - fish are commonly infested by insects.

Indriati *et al.* (1985) reported that the insect most frequently found in processed fish were beetles (*Dermestes* and *Necrobia* spp.), flies (*Chrysomyia* and *Piophila* spp.) and mites. However, information on the extent of damage to processed fish is limited. Tazwir and Indriati (1991) reported the types of damage and physicochemical condition of dried fish resulting from insect infestation in various retail markets in Jakarta. They showed that the infestation occurred mainly in the small rather than large retail markets and wholesale warehouses. This condition was associated with poor physical conditions of storage and extended time of the product held at small retailers. Anggawati *et al.* (1991) found that dried unsalted anchovies were commonly attacked by mites while *Dermestes* spp. were more frequently found on dried fish with low salt content.

In the common traditional practice, insect infestation of dried fish are difficult to control during processing, storage, transportation and marketing because of a lack of knowledge of environmental conditions that influence the susceptibility of the fish to insect attack. However, if the conditions which favour individual pest species can be defined, then appropriate interventions can be made to minimize pest effect.

The objectives of the study are:

- Define when and where dried fish are attacked.
- Assess losses due to insect infestation at different stages of production, distribution and marketing of dried fish.
- Monitor physicochemical changes of fish which affect the susceptibility of fish to insect infestation during distribution and marketing.

MATERIALS AND METHODS

Observations of insect infestation were made on all kinds of dried fish at several sites, warehouses and stores. Estimation of the extent of the infestation was carried out according to the method described by Indriati *et al.* (1985) by levels of infestation within boxes or cartons of fish are estimated by the examination of fish in the upper four layers. Interviews were also carried out with the processors, distributors, retailers and vendors using questionnaires seeking their method of control and the consumers response to infested dried fish.

Samples of dried fish were also taken for analyses of water content, salt content, water activity and degree of infestation at the Research Station Marine Fisheries laboratories. Insects present in the samples were identified originally under a stereomicroscope using the pest identification key of Haines and Rees (1989). Environmental conditions such as air temperature, humidity, lighting and air flow in storage/display rooms were also recorded.

The data were analyzed according to the stages and regions in the marketing chain.

The research was carried out in Jakarta and provinces of West Java from April to May 1991. The samples were drawn from processors, distributors and retailers in each location as described by Wibowo *et al.* as indicated in Table 1.

Table 1 Market sites and their locations with numbers visited

Type of business	Location		No. of samples
Processors	Jakarta	: Muara Angke	5
	Cirebon	: Pelabuhan	2
	Indramayu	: Pabean Udik	1
Distributors	Jakarta	: Bandengan	3
	Bandung	: Pasar Andir	3
	Sukabumi	: Pasar Pelitak : Pasar Cibadak	4
Large Retail Stores	Jakarta	: Pasar Senen, Jembatan Dua, Kalibaru	8
	Bandung	: Pasar Andir	4
	Sukabumi	: Pasar Pelita, Cisaat, Cibadak	10
	Bogor	: Lawang Seketeng, Luewiliang	9
	Cirebon	: Pasar Pagi, Pasar Pekalipan	4
Small Retail Stores	Jakarta	: Pasar Bendungan, Pasar Minggu, Ps. Palmerah	8
	Bandung	: Pasar Andir	3
	Bogor	: Pasar Leuwiliang	4
	Indramayu	: Pasar Indramayu	3
	Sukabumi	: Pasar Cisaat	5

RESULTS AND DISCUSSION

The susceptibility of dried fish to insect attach from processing to retail sale.

The problems encountered by fish processors, distributors and retailers are given in Table 2 which indicates levels of damage at various stages from dried fish production through marketing to consumer.

Table 2 Types of damage observed at various sites associated with dried fish production and marketing

Type of damage	Degree of damage at location of			
	Processors	Wholesale distributors	Large retail stores	Small retail stores
Browning	-	+	+ +	+ +/+ + +
Red halophilic bacteria	-	+	+ +/+ + +	+ +/+ + +
Moulds	-	-	+	+ +
Insects:				
Chrysomyia	+ +	-	-	+/+ +
Piophila	-	+	+ +/+ + +	+ +/+ + +
Necrobia	-	+	+ +	+ +
Dermestes	-	-	+ +	+/+ +
Mites	-	-	+ +	+/+ +

Note:	
-	no damage
+	low (<10%, <10 damaged fish/package)
+ +	moderate (10-50%, 10-50 damaged fish/package)
+ + +	high (>50%, >50 damaged fish/package

It was found that the extent of damage at certain locations was determined not only by the bad condition of the incoming products, but also by the manner in which the owners handled them. Generally, distributors and retailers can, by long experience, predict whether the product can rapidly spoil or be stored longer to obtain a better price.

Insect infestation of dried fish

The processors major concern is the blow-fly (*Chrysomyia megacephala*), which is commonly found during processing of thick fleshed fish such as semi-fermented salted marine catfish. The infestation occurs especially during the wet season when drying takes a considerably longer time (2-3 days). At these processing sites, the larvae of *Chrysomyia* can either grow on fish or in contaminated brine with a salt content of 15-20 percent. The flies attach fish with a moisture content higher than 65 percent and lay their eggs on the flesh. Soon after hatching, the larvae attack the flesh causing black discolouration of the flesh.

Small flies (*Piophila casei*) attack fish that have been incompletely dried or become wet during extended storage, distribution and marketing. During observation at the end of wet season, *Piophila* larvae were found at all locations although the extent of attack varied. These flies are attracted to fermented products, with salt content less than 10 percent and a moisture content higher than 30 percent, producing an odour of butyric acid. The larvae burrow inside the gill and belly cavity of the fish. After feeding they emerge and metamorphose to pupae on the skin of the fish. Such infested fish have a very unattractive appearance.

Small black beetles (*Necrobia*) were found at all locations and in all kinds of fish. Unlike hairy dermestid larvae which crawl on dried larvae, the smaller *Necrobia* larvae are usually found at the bottom of package. The adults are predators of dermestid larvae and they may also consume moulds present on the fish (FAO, 1981). *Necrobia* larvae also eat dried fish and live in the softer parts of the fish flesh. They occur in low numbers on fish and as they are known to be slow growing, their presence on fish is not seriously considered to be destructive.

Dermestes spp. were found in poorly handled fish stored in dark and dirty locations. Dermestid larvae were usually found hiding within layers of fish at the bottom or between insulating papers of wooden boxes because they dislike illuminated conditions. There were several species found at selling places namely *Dermestes maculatus, D. carnivorus, D. frischii* and *D. ater.* The first two species are the most dominant species. Dermestids were found on fish with moisture content less than 20 percent and salt contents less than 10 percent such as unsalted dried anchovies and smoked fish.

Losses due to insect infestation

Blow-flies (*C. megacephala*) cause no significant weight loss, but a financial loss of up to 50 percent was recorded due to their presence. The infested fish will be considered as the lowest standard by consumers. In addition to semi-fermented salted marine catfish, *Chrysomyia* also attacks slightly wet, thick fleshed fish such as Spanish mackerel and shark during marketing.

Infestation by *Piophila* causes high losses. In a short period of time, the infested fish will become covered by pupae and consequently the fish are classified as of very low quality and only marketable as animal feed. A price reduction from Rp. 2,000 to Rp. 100/Rp. 200 per kg was recorded in this survey.

Mites cause a considerable loss of unsalted dried anchovies and small shrimp. They were found at all sites where dried fish were stored for longer than a month especially in the early wet season. Weight losses suffered by the stored fish at the wholesale level were approximately one to three percent but at the retail stage losses reached 40 percent. In the extreme atmospheric storage, i.e. relative humidities >70 percent, a price loss of 75 percent was recorded as the product was marketed as animal feed.

Losses due to dermestid larvae attach are relatively high and within two weeks the larvae can destroy 75 percent of unsalted anchovies. These infested fish were priced at a very low level due to the severe physical damage of breakage and fragmentation.

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Level of insect infestation

The number of fish damaged by insects at a particular place is influenced by the environmental conditions of site and attitude of the owners toward the presence of infested fish. The quality of fish being stored reflects the conditions of storage. A high quality product was generally stored in a clean, tidy room with adequate lighting and ventilation. On the other hand, a poor quality product was most often associated with poor, unhygienic, dark and damp storage.

Piophila infestation causes a most drastic reduction in the quality of stored fish. The presence of larvae and pupae of this insect can very quickly produce the lowest quality fish.

Infestation by other insects (*Dermestes* and *Necrobia spp*) was not generally known or recognized by traders. Mites were not considered as species that caused price reductions.

Data on quantitative losses of dried fish due to insect infestation was difficult to obtain from the owners since the quality of fish varies from one time to another. However, Indriati *et al.* (1985) have shown that levels of infestation by infesting organism were as follows: *Piophila* 21 percent, *C. megacephala* 1.3 percent, *Dermestes* 3.5 percent, mites 3.5 percent and moulds 16.5 percent. It was also shown that environmental, conditions inducing infestations were air temperatures of 28-32°C percent, humidities of 73-87 percent and fish with Aw's of 70-75 percent RH and salt contents of 7-21 percent.

Physico-chemical conditions of infested fish

Generally infested fish are those that have been completely dried or have low salt contents. Insect infestation causes physical damage which brings about various changes such as oxidative rancidity, tissue browning reactions, an increase of Aw, growth of pigmented halophilic bacteria and enhancement of mould growth.

In commercial practice, the quality of dried fish is very much determined by appearance (colour, salt crystals, damage), odour, saltiness, dryness, texture, presence of red halophilic bacteria, insect and moulds. Consumers in urban areas (Jakarta and Bandung) preferably consume fish that is not too salty (salt content <15 percent) such as anchovies, unsalted squids, spiced sardines, sepat (*Trichoquaster sp.*) gabus (*O*phiocephalus striatus) and peda (fermented chub mackerel). However, these products are very susceptible to insect infestation. For this reason the retailers do expect processors to improve the quality of their products.

Table 3 Extent of damage due to insect infestation at different locations and fish marketing sites in West Java, Indonesia - April to May 1991

Location	Damage level (percent)	Processors	Whole-salers	Large retail stores	Small retail stores
Cirebon	0-10	89	-	33	83
	11-25	11	-	67	17
	26-50	0	-	0	0
	51-100	0	-	0	0
Indramayu	0-10	67	-	-	67
	11-25	0	-	-	0
	26-50	11	-	-	22
	51-100	22	-	-	11
Jakarta	0-10	78	100	83	66
	11-25	11	0	10	7
	26-50	11	0	0	27
	51-100	0	0	7	0
Bandung	0-10	0	100	40	67
	11-25	0	0	33	0
	26-50	0	0	20	33
	51-100	0	0	7	0
Sukabumi	0-10	0	100	81	83
	11-25	0	0	9	0
	26-50	0	0	5	0
	51-100	0	0	5	17
Bogor	0-10	0	-	77	100
	11-25	0	-	0	0
	26-50	0	-	6	0
	51-100	0	-	17	0

Insect infestation control

Consumer awareness of the quality of dried fish has forced processors and traders to control insect infestations. In practice there are various methods being implemented to control insect infestations such as insecticides, additional use of salt, improvement of packaging and transportation, increasing turnover by selling goods at lower prices, greater use of chilled rooms, recleaning of fish packs and storage rooms, installation of better lighting and improving air circulation and even redrying products.

Processors

To overcome *Crysomyia* infestations the processors often add pesticides to the brine used for fish salting, or applied by dipping, painting or spraying of the fish prior to drying. The only insecticide recommended for use on fish in markets is Minawet (pyrimiphos methyl). The insecticide was introduced to processors in Indramayu, Cirebon and Muara Angke (Jakarta) in 1985. However, in practice, Minawet is not favoured by the processors because of its relatively high price (Rp. 11,000/can) and lesser effectiveness. Indeed flies are attracted to the treated product. Fish treated by soaking in Minawet at a concentration of 1 ml/litre (0.25 percent active ingredients) did not suppress infestation by flies. For this reason, general domestic insecticides are often used as they are considered to be more effective.

An alternative method of insect control is the use of higher amounts of salt during processing in the wet season as sun-light becomes a limiting factor in the traditional sundrying.

The damage caused to dried fish during transportation can be controlled to a certain extent by improved packaging and protection of the product on vehicles. A better infrastructure for transportation has also contributed to a faster delivery of goods from the processors to the consumers as this reduces the risk of infestation during transportation.

Wholesale

An interesting but impractical method to prevent insect infestation at wholesale distribution point is to increase turnover of the product by selling the commodity at a lower price when it is known to be susceptible to insect infestation. The most reliable method that is commonly employed by some large distributors is the use of chill rooms for storing the product at operating temperatures of 5-10°C. Newly arrived goods are usually displayed in the store for 2-3 days and unsold products are then kept in the chill room overnight. High rates of turnover result in low levels (less than five percent) of infestation.

Large retail stores

The turnover of goods at retail markets is usually high since the products are completely sold within two weeks. The most common insect infestation controls at this point are: 1) storage in chill rooms, 2) sorting infested fish followed by repacking, 3) selling products at a low price and 4) reprocessing (cleaning, washing and drying) during slack periods. When the products are largely low quality fish, no attempt is made to prevent insect infestation.

Small retail stores

Small retailers are usually more concerned with their goods compared to the large retailers. Stores are always cleaned daily and processing as well as other means of insect controls are frequently employed. However, insect infestation occurs at higher level at the small retailers because the turnover of the product is relatively slow (the products cannot be sold completely in less than two weeks). Furthermore, the close proximity of other infested stores provides a continual source of invading insects.

CONCLUSION

Chrysomyia infestation on fresh, drying fish occurs mainly in the processing place when the moisture content of the product is greater than 65 percent, while *Piophilia* and *Necrobia* with moisture contents higher than 30 percent in the large retail stores. Demerstids attack mainly unsalted dried anchovies stored in small retail stores especially products with moisture contents less than 20 percent and salt contents below 10 percent. The amount of damage caused by insect infestation at the processing place is 30 percent, wholesaler 5 percent, large retail stores 60 percent and small retail stores 35 percent respectively.

Methods for the control of insects at processing areas were insecticides, additional salt and good packaging and transportation. At the wholesale level, chilling or rapid selling at a lower price was practised. At large retail stores, chilling, rapid selling, reprocessing and good hygienic practices were common, while at small retail stores selling at lower prices and reprocessing were the more common methods utilized.

Generally infested dried fish have higher moisture contents and water activities than uninfested ones. Infestation paves the way for bacterial and mould growth.

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SHELF LIFE OF SARDINE PROTEIN POWDER IN AIR-PACKED POLYETHYLENE BAG

by

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ABSTRACT

Sardine protein powder was produced by the Prorex machine of the Thai Fisheries Department pilot plant. Fish was dehydrated by heating in oil under reduced pressure followed by de-oiling by solvent extraction and grinding.

For cost minimization, the products were air-packed in 44 micron low density polyethylene bags about 50 grams per bag and kept for 12 months.

During storage, the maximum contents of moisture, fat and ash were 3.55%, 3.30% and 18.15% respectively, whereas the minimum protein was 74.18%. These values showed lower moisture and ash and higher protein compared to the standard established by the Institute of Industrial Standards of Thailand. Thiobarbituric acid value increased from 0.030 to 0.066 mg. malonaldehyde/kg.

Water activity increased as the storage time proceeded, in the range of 0.18-0.42 while the water rehydration values fluctuated between 100 and 160 mg/100 g of product. The sensory evaluations of "Kang liang" using sardine protein powder as the main ingredient were acceptable throughout the whole period of storage.

INTRODUCTION

Sardine is low value, high quantity fish, hence it was chosen as raw material for production trial of protein powder by the Prorex machine (Prorex, 1986). The product concept is to be used for solving protein deficiency problems especially among the low income group. Therefore the packaging and shelf life study is designed to get the lowest cost product and to assess the quality change during one year storage.

MATERIALS AND METHODS

The production of protein powder from sardine was done at the Fisheries Department pilot plant at Bangpoeng, Samutprakarn. The production was done as a batch process using the Prorex machine as shown in the flow chart of Figure 1. cooking and dehydrating of the raw material were done through frying in the heating oil in a stream jacketed kettle under reduced pressure. After oil draining the dehydrated raw material was passed to the oil extracting unit for extraction with hexane. Grinding of the evenly dehydrated low-fat content product gives a final product under 200 mesh.

Packaging and storaging stability study

According to Pruthiarenun (1986), the comparative storage stability study between the LDPE air packed and vacuum packed conditions of FPC type B from sardine showed that through the overall product quality of air packet material was lower than vacuum packed, the level of the overall quality assessments of the product under LDPE air packed was still acceptable after one year according to Codex Alimentarius Commission (1982). Therefore only air packing using low cost LDPE bags was chosen for this study as the sardine protein powder chemical and physical properties were similar to the above mentioned product.

The packages of 50 g of sardine protein powder in LDPE (44 micron thickness) bags were prepared for the 1 year storage study at room temperature (30 ± 2°C, RH 57-80%). Chemical, water activity, some microbiological analyses and sensory evaluation of samples including water rehydration determination were carried out periodically during storage. All packaging and storage stability studies were done at the Fishery Technological Development Division (FTDD).

The previous experiment with sardine FPC type B included detailed microbiological assessment which showed that there was no growth of bacteria during storage and there was no evidence of problems with pathogenic bacteria for the whole year of storage. Therefore only a rough assessment of microbiological quality was included in this experiment.

Analysis and Evaluation

The evaluation of product quality under the specific packing and storage conditions were as follows:

Chemical analyses - protein, moisture ash and fat were determined using AOAC (1980) methods. Water activity was also determined by Thermoconstanter NOVASINA (Switzerland). The rancidity was assessed by determination of malonaldehyde in mg/kg and interpreted as TBA number (Tarladgis, 1960).

Microbiological analyses - total plate count, *Staphylococcus aureus, E. Coli*, Salmonella and mould were determined according to the procedures described by ICMSF (1978).

Water rehydration (water absorbency) - water rehydration was measured using a 40 g sample (w) placed into 100 ml cylinder and observing the volume (a). Water was added up to 100 ml and the mixture was stirred thoroughly and left until no more absorbtion occurred. The final volume of wet sample (b) was measured to calculate the water rehydration as follows:

$$\text{rehydration ratio} = \frac{(b-a)\times 100 \text{ ml}/100 \text{ g}}{w}$$

Acceptability test - product from the trial was incorporated into a Thai recipe with controlled ingredients. A hedonic scoring method described by Howgate (1978) was used in this experiment to elicit the spontaneous opinions of eight to ten panellists on the acceptability of the product (Table 1).

RESULTS

The chemical proximate composition results of the product in LDPE bags kept at room temperature for the whole year period are shown in Table 2. Though the moisture contents increased, the results from the start until the end were still within TISI (1987) Standard. The lowest protein content of the product sample was 74.18% the highest ash content was 18.15% on wet basis (equivalent to 76.10% and 18.49% on dry basis). The highest moisture content at the final stage was 3.55%.

Rancidity in terms of TBA number increased as shown in Figure 2. However TBA numbers were kept very low (0.030-0.060 mg malonoldehyde/kg when compared to the value of freshly prepared fish meal (20 to 30 mg malonaldehyde/kg) (DFO, 1979).

The water activity of the product was in the range of 0.18-0.42 which was sufficient to inhibit the growth of most bacteria and moulds (FAO, 1981). The data could be interpreted as straight line graph ($y=0.007x+0.196$) as has been shown in Figure 3 by the method of analysis of Janthalakana (1980).

The rehydration ratio of the product was in the range 100-136 ml/100 g protein powder as also shown in Table 2.

The microbial analyses results at the start of shelf life study; total plate count (TPC) was 6.3×10^5, *Escherichia coli, Staphylococcus aureus*, and salmonella were not detected, and mould was 100 colonies/g respectively. At the final stage the same microbial analyses were repeated again and the results of TPC was 1.6×10^5. *Escherichia coli, Staphylococcus aureus*, salmonella and mould were not detected.

The sensory evaluation of sardine protein powder incorporated in a Thai recipe "Kang Liang" (Table 3) indicated that the total score of odour, texture, flavour, general appearance and colour score was 35.82 at the first start and decreased to 32.8 at the end. However general appearance, colour, odour, texture and flavour was acceptable throughout one year of storage. (The average of total score decreased from 6.78 to 6.25).

DISCUSSION AND CONCLUSION

In making comparison between the sardine protein powder (S-PP) packed in LDPE bags (44 micron thickness) to sardine FPC type B(S-FPC/B) of the experiments of Pruthiarenun (1986) using the same type of LDPE bags under the same storage condition for one year gives the following conclusions:

- Protein content of S-PP was in the range of 75.14 - 77.95% whereas S-FPC/B was only 65.28 - 67.31%. The main reason for the difference was that the processing method of the Prorex machine could produce the lower moisture and fat contents through the frying and de-oiling steps. During one year shelf life studies the moisture content of S-PP increased from 1.47% to 3.55% whereas S-FPC/B increasing from 4.05% to 9.44%.

- The ash contents of the two products were not different as the results were in the range of 15.96 - 18.15% for S-PP and 16.20 - 16.71 for S-FPC/B.

- TBA number of S-PP was between 0.030-0.066 while S-FPC/B was 27.70-70.33. The difference of the results might be due to the different fat contents of the products, as fat content of S-PP was only 2.25-3.30%. But fat content of S-FPC/B was 4.59-8.71%. Fat contents of the two products were higher at the beginning then gradually decreased during the storaging period. In contrast, the TBA number increased at the final stage. Those changes might not have direct correlation but might be due to the increase of moisture content as the LDPE bag still allowed absorbtion of some moisture in both products. (The moisture content of S-PP increased from 1.47% to 3.55% and S-FPC/B increased from 4.05 to 9.44%). Anyhow, these moisture and fat contents were still acceptable according to the TISI standard (1987) as well as the protein and ash contents of the two products.

- Water activity of S-PP increased from 0.18 to 0.42, low values at which microorganisms would not grow (FAO, 1981). It was much lower than water activity of S-FPC/B (0.32-0.88).

- The microbiological assessments of both products showed no growth of any microorganism during the shelf life storage. Therefore if each product was produced, packed and stored at room temperature under good sanitary conditions, the problem due to food borne microorganisms will not happen.

- The overall sensory average score of S-PP was 7.16 at the beginning, decreasing to 6.48 at the end, whereas S-FPC/B started at 6.66 then decreased to 5.74 at the end. Therefore the acceptability of S-PP was better than S-FPC/B during one year storage.

- This comparison involves different experiments. A direct comparison would give a more precise result.

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Table 1. A sensory scoring sheet used in the experiment\

Product	Score									Reason
Date Items	9	8	7	6	5	4	3	2	1	
General Appearance										
Odour										
Flavour										
Texture										
Taste										

Remarks:
9 = like extremely
8 = like very much
7 = like much
6 = like slightly
5 = neither like nor dislike
4 = dislike slightly
3 = dislike much
2 = dislike very much
1 = dislike extremely

Table 2

Analyses results of sardine protein powder packed in LDPE bags

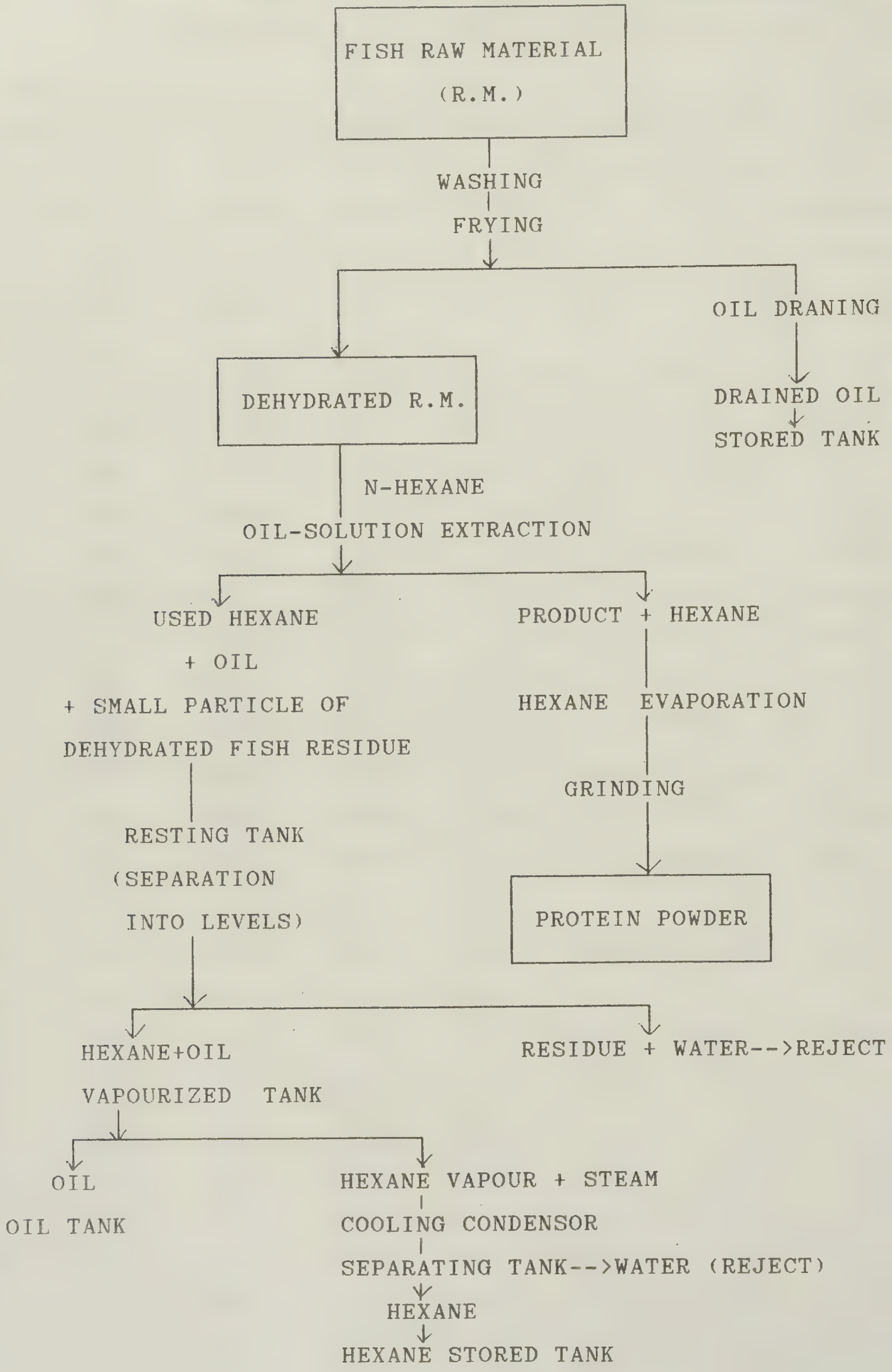
Time (days)	moisture % by wt.	protein % by wt.	fat % by wt.	ash % by wt.	TBA no mg-malonal -dehyde/kg.)	Water activity (Aw)	Rehydration ml/100 g
0	1.47	76.12	3.30	16.99	0.030	0.18	105
6	1.51	76.45	3.17	17.01	0.030	0.20	100
12	1.53	75.65	3.05	18.13	-	-	-
18	1.71	74.18	3.06	17.99	-	-	145
24	1.45	77.29	3.15	15.96	-	-	-
30	1.65	77.95	3.21	16.25	0.031	0.20	-
37	1.82	75.54	2.49	18.15	-	-	160
44	1.27	75.71	2.56	18.15	-	-	-
51	3.06	75.19	2.55	18.01	-	-	152
58	1.79	75.38	2.99	18.13	0.037	0.31	-
66	1.57	76.17	2.69	17.88	-	-	140
72	2.38	76.16	2.40	17.91	-	-	-
79	3.03	76.01	2.00	17.93	0.032	0.20	-
86	2.23	77.56	2.50	17.18	-	-	146
93	2.23	77.53	2.68	16.05	-	-	-
100	2.74	77.50	2.75	16.93	-	-	-
109	2.93	77.13	2.12	17.08	0.048	0.30	132
114	2.46	77.83	2.49	16.46	-	-	128
135	2.41	76.17	2.46	17.04	0.050	0.27	100
157	2.15	77.10	2.58	16.34	0.060	0.30	136
158	2.04	76.33	2.23	17.51	0.055	0.35	120
220	2.57	76.88	2.54	17.89	0.030	0.39	132
262	3.43	75.38	3.00	16.78	0.040	0.40	120
300	3.46	75.14	2.40	17.97	0.066	0.42	128
346	3.55	75.60	2.59	17.12	0.060	0.42	120

Table 3

Sensory evaluation of "Kang Liang" incorporated sardine protein powder (packed in LDPE bags) as main ingredient

Time (days)	general appearance 10 pts.	colour 10 pts.	odour 10 pts.	texture 10 pts.	flavour 10 pts.	Total 50 pts.
0	6.91±1.21	6.78±0.93	7.75±1.42	7.15±0.93	7.23±1.14	35.82
12	6.63±0.92	6.25±1.28	6.13±1.25	6.25±0.89	5.88±0.99	31.14
38	6.75±1.16	6.63±0.74	5.88±1.36	6.13±1.36	6.13±1.13	31.52
67	7.22±0.44	7.00±0.71	6.55±1.01	7.00±0.71	6.66±1	34.43
94	6.89±0.93	6.56±1.01	6.56±1.01	6.44±1.13	6.11±0.98	32.56
136	7.56±0.53	7.33±0.87	7.22±0.44	7.22±0.66	7.04±0.86	36.37
158	7.11±0.93	6.11±1.27	5.89±1.05	6.22±1.09	5.89±0.93	31.22
186	7.22±0.44	7.11±0.60	6.88±0.60	7.00±0.87	6.22±0.67	34.43
221	6.93±0.77	6.11±0.95	5.66±0.86	5.38±1.12	5.60±1.01	29.63
263	6.13±0.35	5.63±0.52	5.13±0.83	4.88±1.13	5.50±0.99	27.27
301	6.63±0.92	6.25±1.28	6.13±1.25	6.25±0.89	5.88±0.9	31.14
347	6.75±0.46	6.63±0.52	6.25±0.71	6.25±0.46	6.50±0.5	32.38

FIG1.FISH PROTEIN POWDER PRODUCTION (BY PROREX MACHINE)


FISH RAW MATERIAL
(R.M.)
WASHING
FRYING
OIL DRANING
DRAINED OIL
STORED TANK
DEHYDRATED R.M.
N-HEXANE
OIL-SOLUTION EXTRACTION
USED HEXANE
+ OIL
+ SMALL PARTICLE OF
DEHYDRATED FISH RESIDUE
PRODUCT + HEXANE
HEXANE EVAPORATION
GRINDING
PROTEIN POWDER
RESTING TANK
(SEPARATION
INTO LEVELS)
HEXANE+OIL
VAPOURIZED TANK
RESIDUE + WATER-->REJECT
OIL
OIL TANK
HEXANE VAPOUR + STEAM
COOLING CONDENSOR
SEPARATING TANK-->WATER (REJECT)
HEXANE
HEXANE STORED TANK

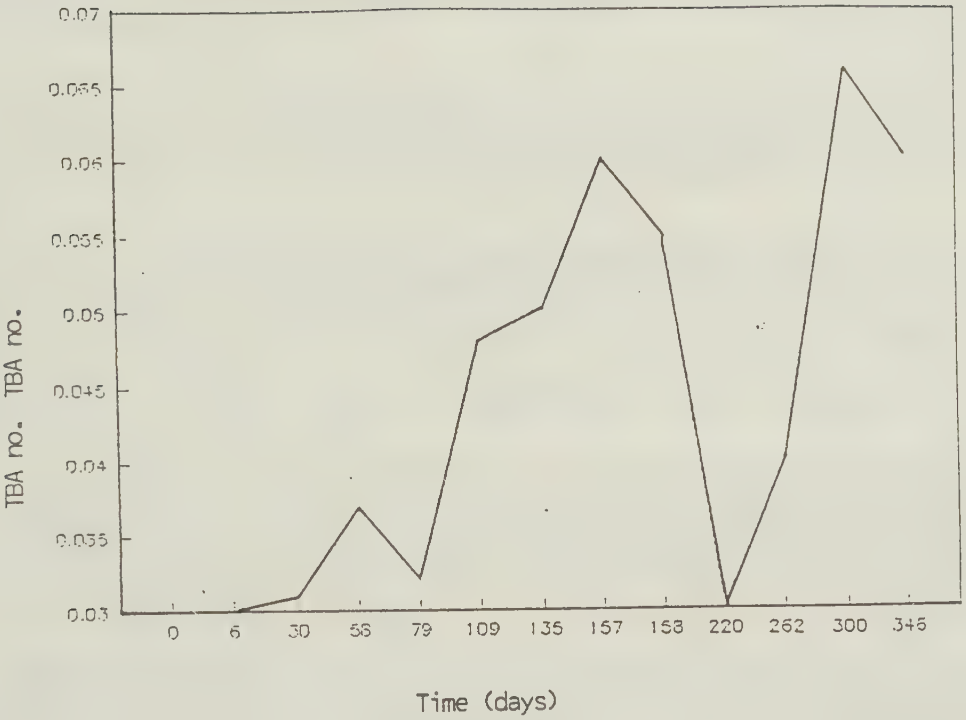


Figure 2. TBA No. of sardine protein powder in LPDE bags during storage

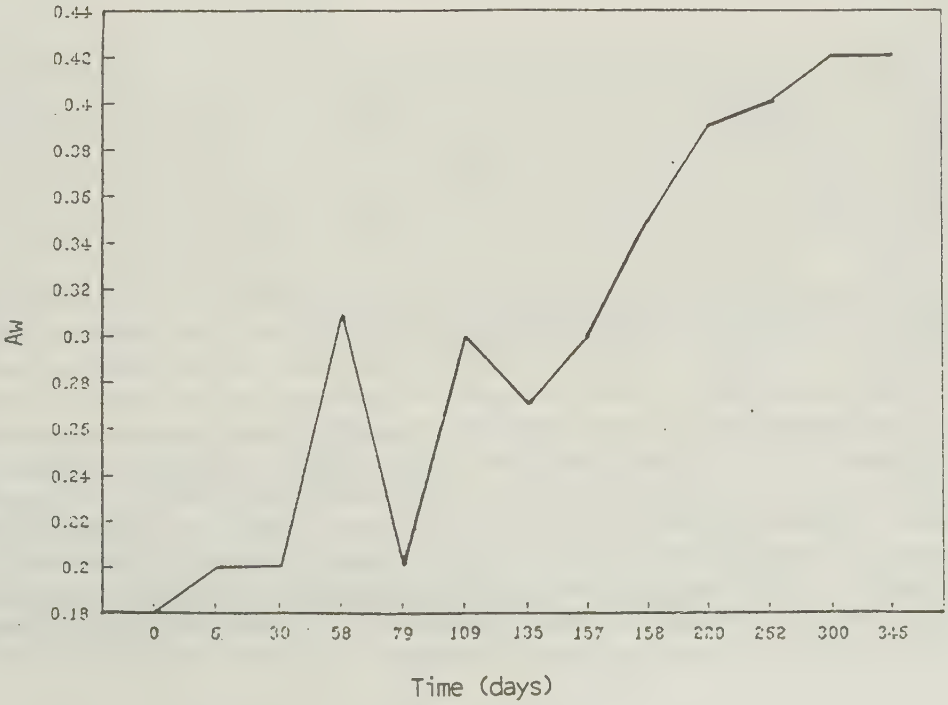


Figure 3. AW of sardine protein powder in LPDE bags during storage

DEVELOPMENT OF HIGH PROTEIN AND CALCIUM RICE-BASED EXTRUDED HEALTH FOOD FROM FISH POWDER

by

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ABSTRACT

Six high protein and calcium snack foods were formulated from three varieties of rice supplemented with by-catch fish powder at the levels of 15 and 18% of total weight. The formulas were extruded in a Brabender Extruder Type 20 DN. The expansion indices of the products ranged from 1.71 ± 0.09 to 2.00 ± 0.09 and the bulk densities ranged from 0.218 ± 0.012 to 0.263 ± 0.017 g/ml. From the six formulated products, the most suitable one was selected for further studies. The selected product contained 20.0 g% protein, 12.1 g% fat, 58.4g% carbohydrate and 821 mg% calcium. It also met the recommended caloric distribution; which the energy contributed from protein, fat and carbohydrate were 19.0%, 25.8% and 55.4%, respectively. The total energy was 422 kcal/100g. The acceptability of the product was 100% in preschool children while the sensory acceptability score was 3.95 + 0.97 on a 5-point face scale in school children. This product could be an alternative source of protein and calcium for consumers who might be allergic to milk or dairy products but need to have better health, better growth and furthermore it could help in prevention of osteoporosis which might occur during the later stage of life.

INTRODUCTION

Thailand is one of the leading food exporting countries but protein-energy malnutrition (PEM) still prevails in some areas of the country. About 18.5% of preschool children suffer from such malnutrition (Wanaratana, 1990) more in rural areas than in urban communities. Without a proper solution those children will continue having the same problem in their school age. At present there are about 17% of school children undernourished. Both groups of children face the same problem which does not only lead to poor physical development and health of individual but also leads to family and national problems with an enormous budget for care and medical treatment (Cravioto *et al,* 1971 and Wanaratana, 1990).

Results from the National Surveys of Dietary Intake and Nutritional Status conducted by the Ministry of Public Health and Faculty of Public Health, Mahidol University in 1986 showed that Thai people had a calcium intake of only 301 mg/person/day which was only 37.63% of RDA, 1989 and osteoporosis was occurred in the aged group (Pisolyabute *et al,* 1989). The importance of an adequate calcium intake at all stages of life is well established. It is a prerequisite for normal bone growth and for the attainment of peak bone mass. Maintenance of an adequate calcium intake is also necessary in the elderly (Dept. of Clinical Chemistry, 1991). A minimum intake of 800 mg of calcium daily is recommended for all adults. Higher amounts are required in adolescence, pregnancy, lactation and old age (Food and Nutrition Board 1989). Milk is a very good source of protein and calcium (Nutrition Div., 1987). However, a report from the National Nutrition Survey in 1986 showed that Thai people consumed only 7.1 g/person/day of milk (Pisolyabute *et al,* 1989), because milk is not part of their traditional diet. Many people in Asia countries are lactose intolerant (Houts, 1988) or allergic to the protein in milk or dairy products (Brunner, 1976) and need alternative sources. Fish contains a high amount of good quality protein and calcium (James, 1984; Young and Bernard, 1988) but unfortunately previous reports showed that fish consumption in Thailand was only 10 kg/person/yr (Nutrition Div., 1986 and Suanpan *et al*, 1988).

An important source of fish that is currently not available for human consumption is by-catch normally coming from trawlers. Large volumes of these fish are presently diverted to the fish meal industries and used as animal feed (Boonjubol, 1984 and Tandberg, 1989). A lot of effort has been made to improve by-catch fish quality for human consumption (Supichayangure, 1985).

Both governmental and non-governmental organizations in Thailand have put a lot of effort in PEM reduction and prevention by using dried ground fish under different names and processing methods incorporated into Thai dishes (Panichpakdi, 1954; Chutima, 1955; Pruthiarenun, 1967, 1984 and 1988 Phithakpol, 1981, 1982 and 1984; Dhanamitta *et al*, 1983; Chindawong, 1984; Veeravaidya, 1984; Suksuchoke, 1985; Winichagoon, 1988; and Charoenphol, 1988). However, fish consumption promotion should be continued with more product development in order to improve palatability and acceptability when higher concentrations of fish are added.

Extruded snack food is a good vehicle for the present study, because it is becoming more and more popular and available in the markets throughout the country. Extrusion cooking is a high temperature short time (HTST) process which minimizes the damage to nutritional quality but at the same time inactivates the growth inhibitors of the product.

The objectives of this study were to (1) investigate the possibility of using by-catch fish powder as a protein and calcium source in rice extruded snack food, (2) evaluate its physical properties, sensory acceptabilities in school and preschool children, and (3) evaluate nutritive values of the product.

MATERIALS AND METHODS

Product formulation

Three varieties of rice (Oryza sativa) represented each group of amylose content i.e. Khao Dawk Mali 105 (low : 12-17% amylose), Khao Tah Hang 17 (slightly high : 26-28% amylose) and Aromatic Pathumthani 60 (high : 27-30% amylose) obtained from Pathumthani Rice Research Center, Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand, were used in this study. Each variety of rice was dehulled and then ground in a hammer mill through a metal sieve 25 mesh (710,μm International standard sieve).

The protein and calcium sources ie. fish powder from by-catch produced by a Prorex machine at the Thai Fisheries Department pilot plant. Fisheries Dept., Ministry of Agriculture and Cooperatives, Bangkok, Thailand; the particle size of the fish powder was smaller than 140 mesh (106 μm International standard sieve). All raw materials were stored at 10°C in sealed plastic bags until used. Soybean oil (Angoon Brand) was used as a source of fat. Iodized salt powder containing 0.01-0.02 g KI/100 g obtained from the Government Pharmaceutical Organization, Ministry of Public Health was also used as an ingredient. However, other flavouring agents including roasted chicken flavour (TSD 0184-A Saroline), cuttlefish flavour (TSD 0064 Saroline) and sweet paprika (TSD 0021-A Saroline) obtained from Bush Boake Allen Company Ltd., Bangkok, Thailand were also used for seasoning the products; the flavouring agents were stored at 10°C in a sealed aluminium foil bag until use.

The raw ingredients (rice, fish powder and salt) were mixed at the ratio based on recommended energy distribution (Kinsella, 1988 and Motulsky, 1989).

Proximate analysis

Proximate composition including moisture, protein, fat, ash and dietary fibre contents were determined by the following methods:-

- Moisture content was determined by drying a sample in a hot air oven at 105°C (Williams,1984).
- Protein content was determined by the Kjeldahl method (Williams, 1984) with a protein conversion factors of 5.95 for rice and 6.25 for fish powder.
- Fat content was determined using Soxhlet extraction after acid digestion (acid hydrolysis) (Sirichakwal *et al,* 1985).
- Ash content was determined by incinerating a sample at 450°C (Pearson, 1976).
- Dietary fibre content was determined by the enzymatic gravimetric method of Prosky *et al,* 1984 with slight modifications (Williams, 1984).
- Carbohydrate content was then calculated by subtracting the percentage of moisture, protein, fat, ash and dietary fibre from 100.

Production of high protein and calcium rice-based snack foods by extrusion process

Moisture contents of the feed mixtures were raised to 16% by adding a certain amount of water and then preconditioned overnight in a 10°C cold room. On the next day, the mixtures were fed at a speed of 10 kg/h into the hopper of a Brabender Extruder type 20 DN which was assembled and operated under the conditions suggested by Boonyasirikool in 1979.

The set operating conditions including screw compression ratio at 4:1, screw speed at 180 rpm, temperature of the heating zones : 1st, 2nd and 3rd zone at 125°C, 170°C and 180°C respectively and air pressure at 10 psi. The mixture was finally extruded through a die orifice size 5 mm in diameter to become extrudates with an average of 4.32 cm in length.

After that the extrudates were dried in a hot air oven at 100°C for 20-30 minutes to reduce the moisture content to less than 5% and then sprayed with cooking oil at the level of 10% by weight. The extrudates were again dried in the hot air oven at 100°C for 15-20 minutes, air cooled, and packed into sealed polypropylene bags until use.

Physical properties evaluation

The plain extrudates were then evaluated for their physical properties i.e. (1) moisture contents by the method of AOAC (Williams, 1984); (2) expansion index by dividing extrudate diameter by extruder die orifice diameter (Gomez *et al,* 1988) with ten replications; and (3) bulk density by sesame displacement method (Harper, 1981) with five replications.

Flavouring process

The factors including results of physical properties, protein content, cost and availability of raw materials were used as the criteria for a selection of one formula to be seasoned with the flavouring agents (1% by weight) and icing sugar (10% by weight); then the flavoured products were dried in a hot air oven at 100°C for 15-20 minutes, cooled and packed in sealed polypropylene bags until use as shown in Figure 1.

These finished products were then evaluated for sensory acceptabilities in adults. The most acceptable flavour was evaluated for its (1) nutritive values including proximate composition by the methods mentioned above and calcium content by the method of AOAC (Williams, 1984) and (2) sensory acceptabilities in school and preschool children.

SENSORY ACCEPTABILITY TESTS

Adults

A nine-point hedonic scale (1, dislike extremely 5, neither like nor dislike, and 9, like extremely) was used to evaluate the acceptability of the products, whereas 5-point just-about-right scale (1, much too weak; 3, just about right; and 5, much too strong) was used to evaluate the suitabilities in colour, odour and taste of the products and 5-point just-about-right scale (1, much too soft; 3, just about right; and 5, much too hard) was used to evaluate the suitabilities in crispiness of the products (Stone and Sidel, 1985). However, Randomized Complete Block (RCB) design was used for the design of sample presentation (Uppadisakool, 1990). Forty-three adults: 14 men and 29 women aged 20-45 years old from the Faculty and staff of the Institute of Nutrition, Mahidol University were used in the panel. The test was performed at the Sensory Science Laboratory of the Institute. Each panellist obtained four different tastes of the products i.e. sugar, roasted chicken, sweet paprika and cuttlefish. The data was then analyzed by Analysis of Variance (ANOVA) with the Least Significant Difference (LSD) comparison using SPSSX software.

School Children

The most acceptable flavoured product was then evaluated in school children by using 5-point face scale ranging from 1, dislike a lot; 3, neither like nor dislike; to 5, like a lot (Stone and Sidel, 1985). There were altogether 113 school children; 55 boys and 58 girls aged 6-15 years old at Wat Nong Sanom School, Rayong Province, Thailand.

Preschool Children

Sensory acceptability test of the same product in preschool children was performed by observing and interviewing 117 children; 63 boys and 54 girls aged 4-5 years old at the Child Nutrition Center, Wat Bangkhan Community, Klong Noeng District, Pathumthani province, Thailand. Percentage of the children who like or dislike the product including some comments were recorded.

RESULTS AND DISCUSSION

Product formulation

Table 1: Chemical composition of raw materials

Raw material	Chemical Composition			
	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)
Rice (Khao Dawk Mali 105)	11.7	6.9	1.4	77.6
Rice (Khao Tah Hang 17)	11.5	5.6	1.2	79.9
Rice (Pathumthani 60)	11.1	8.7	1.2	77.0
Fish powder (by-catch)	5.1	77.2	1.6	-

As shown in Table 1, protein content of rice varieties varied widely from 5.6-8.7; however, there were still in the range which had been reported before (Juliano, 1979, Nutrition Division, 1987; Kongseree, 1989 and Nutrition Division, 1991). Aromatic Pathumthani 60 contained the highest amount of protein. Though, rice was not expected to be a main source of protein in this type of product; it could, however, contribute a certain amount of protein as well. The protein content in fish powder was in the same range as reported by Phithakpol (1985). Fish powder was the main source of protein.

Rice could be a low cost carbohydrate source being nearly 80% of total weight, whereas fish powder has no carbohydrate. Because the moisture contents of raw materials were below 16%, some more water was added in order to optimize the moisture level of feed ingredients. All raw materials had low fat contents as shown in Table 1, therefore fat must be supplemented.

Fish powder was not only high in protein but also in calcium (4,386 mg%) which was higher than the previous reports (Norsildmel 1980 and Phithakpol, 1985). This was because fish powder used in this study was made of by-catch fish with a high bone to flesh ratio.

Normally this type of fish powder was not accepted well when incorporated into traditional recipes, due to poor functional properties, low palatability, sandiness and darkness. It had a very strong fishy odour because it made of whole fish. So in the past, by-catch fish powder was mainly used as fertilizer and fish meal (Boonjubol, 1985 and Tandberg, 1989). The present study formulated new products from by-catch fish powder by extrusion process for human consumption.

Calculations based on energy distribution gave 6 formulas from 3 varieties of rice with 2 levels of fish powder as shown in Table 2, and the energy distribution as shown in Table 3. Energy from protein, even at a lower level of fish powder (15%), was still higher than 15% kcal in all formulas, but it cost less when compared to the 18% of fish powder level formulas (Table 4). However, all formulas had the same range of total energy (Table 3).

Table 2 Ingredients of high protein and calcium extruded snack foods

Formula	Ingredient					
	Rice*			Fish powder	Oil	Salt
	KDML105 (%,w/w)	KTH17 (%, w/w)	PT60 (%, w/w)	(%, w/w)	(%, w/w)	(%,w/w)
1	73	-	-	15	10	2
2	70	-	-	18	10	2
3	-	73	-	15	10	2
4	-	70	-	18	10	2
5	-	-	73	15	10	2
6	-	-	70	18	10	2

* KDML105 = Khao Dawk Mali 105
KTH17 = Khao Tah Hang 17, and
PT60 = Pathumthani 60

Table 3 : Energy distribution of the snack foods

			Energy distribution		
Formula	Ingredient*	Total energy (kcal/100g)	Protein (%kcal)	Fat (%kcal)	Carbohydrate (%kcal)
1	KDML:FP:Oil:salt (73:15:10:2)	424	16.8	25.7	57.5
2	KDML:FP:Oil:salt (70:18:10:2)	423	19.0	25.8	55.2
3	KTH:FP:Oil:salt (73:15:10:2)	426	15.8	25.3	58.9
4	KTH:FP:Oil:salt (70:18:10:2)	425	18.0	25.4	56.6
5	PT:FP:Oil:salt (73:15:10:2)	426	18.1	25.2	56.7
6	PT:FP:Oil:salt (70:18:10:2)	426	20.2	25.3	54.5

* KDML = Khao Dawk Mali 105
KTH = Khao Tah Hang 17
PT = Pathumthani 60
FP = Fish powder.

Physical properties evaluation

The plain extrudates contained below 5% moisture content as shown in Table 4; they should therefore have good storage properties (Ministry of Public Health, 1985).

Expansion, as measured by expansion index and bulk density, was used to express product characteristics after extrusion. The relationship between the expansion of extrudates and protein, and types of cereals with different amount of amylose is presented in Table 4.

Table 4 Physical properties protein content and cost of raw ingredients of the extrudates

Formula	Ingredient[2]	Moisture (%)	EI[3]	Bulk density	Protein (%, db)[4]	Cost[5] (Baht/kg)
1	KDML:FP:Oil:salt (73:15:10:2)	4.4	1.78 ± 0.06^{c}	0.257 ± 0.019^{a}	18.7	32
2	KDML:FP:Oil:salt (70:18:10:2)	4.5	1.71 ± 0.09^{d}	0.263 ± 0.017^{a}	21.1	36
3	KTH:FP:Oil:salt (73:15:10:2)	4.4	1.98 ± 0.04^{a}	0.220 ± 0.022^{c}	17.6	32
4	KTH:FP:Oil:salt (70:18:10:2)	4.4	1.92 ± 0.08^{b}	0.239 ± 0.016^{b}	20.0	35
5	PT:FP:Oil:salt (73:15:10:2)	4.2	2.00 ± 0.09^{a}	0.218 ± 0.012^{c}	20.1	32
6	PT:FP:Oil:salt (70:18:10:2)	4.4	1.89 ± 0.09^{b}	0.222 ± 0.007^{b}	22.5	35

[1] Means with the same letter within a column are not significantly different ($p > 0.05$)

[2] KDML Khao Dawk Mali 105, KTH = Khao Tah Hang 17, PT = Pathumthani 60 and FP = Fish powder.

[3] EI expansion index

[4] db dry basis

[5] Cost of raw ingredients

The values of expansion index and bulk density depended on protein and amylose contents of the extrudates. Table 4 showed that expansion indices of the extrudates ranged from 1.71 ± 0.09 to 2.00 ± 0.09, which were good values as classified by Trishnamurti *et al*, 1980.

In the present study, it was found that the higher protein content of the same variety of rice reduced the expansion index (proportional to the reciprocal of bulk density) as shown in Table 4. Only the extrudates of Khao Dawk Mali 105 with a different amount of protein, showed no significant difference ($p > 0.05$) in bulk density. The results were similar to Manoharkumar *et al.* (1978) who used plant protein at 12% moisture level of the feed mixtures.

Pathumthani 60 was selected based on criteria of protein content, cost, expansion index, bulk density and availability for further studies.

Sensory acceptability tests

The results of sensory acceptability test of rice product with four different flavours in 43 adults showed that roasted chicken flavour was the most accepted in overall acceptability and all other suitability attributes (Table 5). The same flavoured product was also accepted well in 113 school children (3.95 ± 0.97) and preschool children (100% like). Fish powder in this type of product did not lower the product acceptability as it did in other types of products studied in the past (Dhanamitta *et al,* 1983).

Some panellists commented that the characteristics of the products was similar to traditional shrimp chips; this might result in the high acceptability of the product.

Nutritive values

The nutritive values of the roasted chicken flavoured rice product are shown in Table 6. Protein content of this product is higher than that of commercial extruded snack foods available in the markets as reported by Ang, *et al,* 1980; Sinthavalai, 1984; and Boonyasirikool, 1986.

The product also met the recommended caloric distribution mentioned by Kinsella (1988) and Motulsky (1989). One package (25 g) of this product a day could help supplement daily consumption in order to partially fulfil RDA for protein and calcium as shown in Table 7.

Table 5 Means and standard deviations of the sensory acceptability scores of rice product with different flavours on adult[1]

Flavour	Overall acceptability[2]	Colour[3]	Odour[3]	Taste[3]	Crispiness[4]
Sweet	5.75 ± 1.48^{b}	2.10 ± 0.50^{b}	2.52 ± 1.01^{b}	2.80 ± 0.76^{a}	3.10 ± 0.50^{ab}
Roasted chicken	6.43 ± 1.39^{a}	2.82 ± 0.70^{a}	3.05 ± 0.84^{a}	2.90 ± 0.71^{a}	2.98 ± 0.42^{ab}
Sweet paprika	5.38 ± 1.51^{b}	2.90 ± 0.54^{a}	2.85 ± 0.83^{ab}	2.38 ± 0.81^{b}	2.90 ± 0.54^{b}
Cuttlefish	5.90 ± 1.56^{ab}	2.85 ± 0.74^{a}	3.15 ± 0.97^{a}	2.45 ± 0.78^{b}	3.18 ± 0.50^{a}

[1] Means with the same letter within a column are not significantly different ($p > 0.05$)

[2] Overall acceptability used a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely)

[3] Colour, odour and taste used a 5-point just-about-right scale (1, much to weak; 3, just about right; 5, much too strong)

[4] Crispiness used a 5-point just-about-right scale (1, much too soft; 3, just about right;5,much too hard)

Table 6: Nutritive values of rice product, per 100 g

Energy (kcal)	422
Protein, g	20.0
% of total calories	19.0
Fat, g	12.1
% of total calories	25.8
Carbohydrate, g	58.4
% of total calories	55.4
Calcium, mg	821

Table 7 Nutritive values of rice product (25 g package) compared to RDAS, 1989 (Food and Nutrition Board, 1989).

Subject (yr)	% RDA	
	Protein	Calcium
4-6	20.8	25.7
7-10	17.9	25.7
25^{+}, men	8.2	25.7
25^{+}, women	10.0	25.7

CONCLUSION

Extruded snack food could be a good vehicle for protein and calcium from by-catch fish powder in order to get better health, better growth in the younger generation and osteoporosis prevention in adults.

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DEVELOPMENT OF FISH BISCUITS FROM ROUND SCAD
(*Decapterus Russelli Rupp.*)

by

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ABSTRACT

Fish biscuits were successfully developed using the marine fish *Decapterus russelli Rupp.*. Two types of flour, (low and high protein), two methods of fish mince preparation (water-heated and oven-dried), and three levels of fish (8%, 16% and 24%) were tested in the formulations. Proximate analyses, water activity and colour measurements were carried out. Results from sensory evaluation showed that the most acceptable formulation for the biscuit was obtained using 16% of water-heated fish mince and low-protein flour.

INTRODUCTION

In Malaysia, fish - particularly marine fish - is the main source of dietary protein, apart from being the cheapest. In 1987 an increase of 32.5% of marine fish landings was reported, as compared to the previous year in Peninsular Malaysia. Of this, pelagic fish showed an increase of 80% from the previous year. One such species is round scad (*Decapterus russelli Rupp*). A landing of 40,652 metric tonnes of this fish was reported in 1987.

Of the many species of fish landed off the East Coast of Peninsular Malaysia during the monsoon season, round scad is one of the most underutilized.

This species has a relatively high oil content, dark flesh, strong flavours and odours and tends to deteriorate rapidly after catch. These characteristics explain why the species is generally not used much for food. In addition, the oil content is too high to make processed products such as dried and salted products. It is not an uncommon sight to see piles of round scad rotting on jetties or by the seashore.

Development of new fish products from this species could not only contribute to supplementing the diet of some of needy children in this part of the country, in which the economic situation is less favourable but also provide added economic value for the species especially for the fishermen along the East Coast of Peninsular Malaysia. The new product should be in form familiar to, and readily acceptable by, the population, particularly the children and be easy to transport and store in a village environment. Biscuits fortified with round scad would fulfil the above requirements and would therefore be a suitable product for such development.

MATERIALS AND METHODS

Preparation of the Leached Fish Mince (Figure 1)

The fish was purchased fresh from the wholesale market in Kuala Lumpur and transported in ice to the laboratories. Gutting was done manually and the flesh separated using a mechanical deboner. The mince was first washed in 0.2% salt solution (1 part fish mince : 3 parts salt solution at 5-10°C). Excess water was centrifuged out (Ban Hing Weights & Measures, Kuala Lumpur) at 200 x g through a muslin cloth. The second washing was carried out using 0.3% NaCl (1:2 parts of fish mince to water) and excess water was removed by centrifugation.

The fish mince was then cooked using two methods: heating in water and oven-drying. Heating in water was done at 95-97°C in a jacketed steam heater (Groen, Germany) for 10-15 minutes (1 part mince : 3 parts water). Excess water was expressed through muslin and the dried mince packed in polyethylene bags (0.08 mm thickness) and stored at -20°C. Oven-dried fish mince was prepared by spreading the leached mince on to trays to 2mm thickness and drying at 100°C in a continuous flow oven (Memmert UL80, Germany) for two hours. The dried mince was blended (National Superblender, MX-MG 200N, Japan) into a powder, packed in laminated aluminium foil and stored at -20°C.

Preparation of the Fish Biscuits (Figure 2)

The ingredients were mixed in a chilled bowl cutter (ADE SL18 Hamburg, Germany). Two types of wheat flour (Balance Brand and Diamond Brand) as recommended by Malayan Flour Mills Bhd. and three levels of fish mince (8%, 16%, 24%) were used in the formulations. Other ingredients used are shown in Table 1.

The dough was sheeted between rollers (Ban Hing Weights and Measures, Kuala Lumpur) to a thickness of 1 mm, cut into dimensions of 5.0 cm x 5.0 cm and baked in a Rotator Oven (Rotator Sveba Ultra, Sweden) at 175°C for 12.5 - 15 minutes.

Sensory Evaluation

A panel of 21 panellists were used to evaluate the colour, texture, flavour, odour and overall acceptability of the biscuits on a 7-point Hedonic Scale (Larmond, 1977). Two batches, each made from different flours, were presented and the Duncan's Multiple Range Test (O'Mahony, 1986) was used to evaluate the best formulation from each batch. The best biscuit from each batch was then compared in a paired-preference test (O'Mahony, 1986).

Proximate Analyses

The protein, fat and moisture contents were estimated according to Pearson (1976). Water activity was measured (Novarina AG Model EE JA 3, Switzerland) using sodium chloride and magnesium nitrate and chloride as standards. Colour measurement was carried out using the Hungerlab Colorimeter D 25 L-2.

RESULTS AND DISCUSSION

Organoleptic Acceptability

For both types of flour, biscuits with the water-heated fish mince were generally preferred (Tables 2 and 3). The water heated fish mince used was also lighter in colour compared to oven-dried fish mince, due to the exposure of the latter to heat for a longer period of time (Table 4). Subsequently, biscuits made from water heated mince were lighter in colour.

For fish biscuits made from Diamond Brand Flour, there was little difference between samples containing 16% and 24% water heated fish mince. The latter sample was chosen for the paired preference test as its overall acceptability scores (Table 2) and protein content (Table 6) were higher. For samples with Balance Brand Flour, there were no significant differences between the 16% and 24% water heated fish mince sample in terms of odour, flavour and overall acceptability. However, colour and texture in the 16% sample were preferred (Table 3). Hence, this sample was chosen for the paired preference test.

The paired-preference test comparing these two samples showed that the biscuit made from Balance Brand Flour with 20 g water heated fish mince was preferred. 27 panellists out of 30 chose it over the biscuit made from Diamond Brand Flour, containing 30 g water heated fish mince (Table 5). Panellists commented that the preferred sample had a better mouthfeel, was crisper and had a pleasant fishy taste (Figure 3).

In the production of biscuits and crackers, a weak flour which produces a dough with pronounced plastic properties is preferred. Of the two types of flour used, Balance Brand Flour had a lower protein content (Table 6) in the range of normal flours used for biscuits (7-9%) (Wade, 1988). Biscuits produced from Balance Brand Flour were therefore more acceptable to taste panellists.

Proximate Analyses

The protein content (Table 6) of the fish biscuits was much higher compared to commonly-consumed biscuits in Malaysia. A survey by Tee *et al.* (1988) showed that most Malaysian biscuits contain less than 10% protein. A low moisture and A_w values (0.21) enhance keeping quality. Although the fat content is high, storage in sealed polyethylene bags at ambient temperatures (26°C) for 8 weeks produced no detectable changes as determined by sensory evaluation. The high fat content is necessary to produce a dough of workable consistency and a more tender-eating product. Studies on longer storage periods are currently the subject of further research.

CONCLUSION

A formulation for fish biscuit from *D. russelli Rupp.* has been successfully developed. Response from in-house taste panels were very encouraging and preliminary large scale sensory evaluation trials show good response (Yu - unpublished data). A training course in fish biscuit production has been planned for November 1991 for biscuit processors from the East Coast of Peninsular Malaysia. The acceptability of the fish biscuits would increase the economic value of *D. russelli Rupp.* and also supplement the diet of needy children along the East Coast of Peninsular Malaysia.

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Mention of any company or product does not constitute endorsement.

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Table 1 Formulation for Fish Biscuits

Ingredients	%
Vegetable fat (solidified palm oil)	11.0
Sugar	2.3
Salt	1.4
Ammonium bicarbonate	0.5
Sodium bicarbonate	0.3
Monosodium glutamate	0.9
Pepper	0.4
Chilli powder	0.2
Water	21.0
Fish	8% 16% 24%
Flour	54% 46% 38%

Mean values within a column followed by a common alphabet are not significantly different at 5% level of probability.

Table 2 Organloeptic Acceptability of Fish Biscuits with Diamond Brand Flour

Biscuit Sample	Mean values				
	colour	texture	odour	flavour	overall acceptability
With water-heater fish mince					
24%	5.5^{a}	5.4^{ab}	4.9^{a}	4.9^{a}	5.4^{a}
16%	5.4^{a}	5.9^{a}	4.7^{a}	4.9^{a}	5.1^{a}
8%	4.6^{ab}	2.9^{c}	4.4^{a}	4.0^{a}	$3.7b^{c}$
With oven-dried fish mince					
24%	4.0^{bc}	5.5^{ab}	4.3^{a}	4.2^{a}	4.6^{a}
16%	3.2^{c}	3.6^{c}	4.1^{a}	4.1^{a}	3.8^{b}
8%	4.7^{a}	4.9^{b}	4.7^{a}	4.6^{a}	4.9^{a}

Table 3 Organloeptic Acceptability of Fish Biscuits with Balanced Brand Flour

Biscuit Sample	Mean values				
	colour	texture	odour	flavour	overall acceptability
With water-heater fish mince					
24%	5.7^{ab}	5.9^{ab}	4.8^{a}	4.9^{a}	5.4^{a}
16%	6.2^{a}	6.0^{a}	4.7^{a}	5.1^{a}	5.1^{a}
8%	4.4^{c}	2.6^{c}	3.8^{a}	3.3^{c}	3.3^{c}
With oven-dried fish mince					
24%	3.3^{d}	5.1^{b}	4.7^{a}	4.3^{a}	4.4^{b}
16%	4.6^{c}	4.5^{b}	4.7^{a}	5.0^{a}	5.2^{ab}
8%	4.7^{bc}	4.9^{b}	5.0^{a}	5.1^{a}	5.2^{ab}

Mean values within a column followed by a common alphabet are not significantly different at 5% level of probability.

Table 4 L, a, b Values of Fish Mince

Sample	L	a	b
Water-heated mince	37.7	+1.7	+11.3
Oven-dried fish mince	36.4	+4.0	+14.0

Table 5 Results of the Paired-Preference Test

Total number of panellists	30
Panellists preferring sample with Balance Brand Flour	27

Table 6 Proximate Analysis of Wheat Flour and Fish Biscuits

Sample	%			
	Moisture	Ash	Protein	Fat
Fish Biscuit	0.71	0.26	21.50	23.9
Diamond Brand Flour	14.25	0.18	13.20	Nil
Balance Brand Flour	13.48	0.21	9.63	Nil

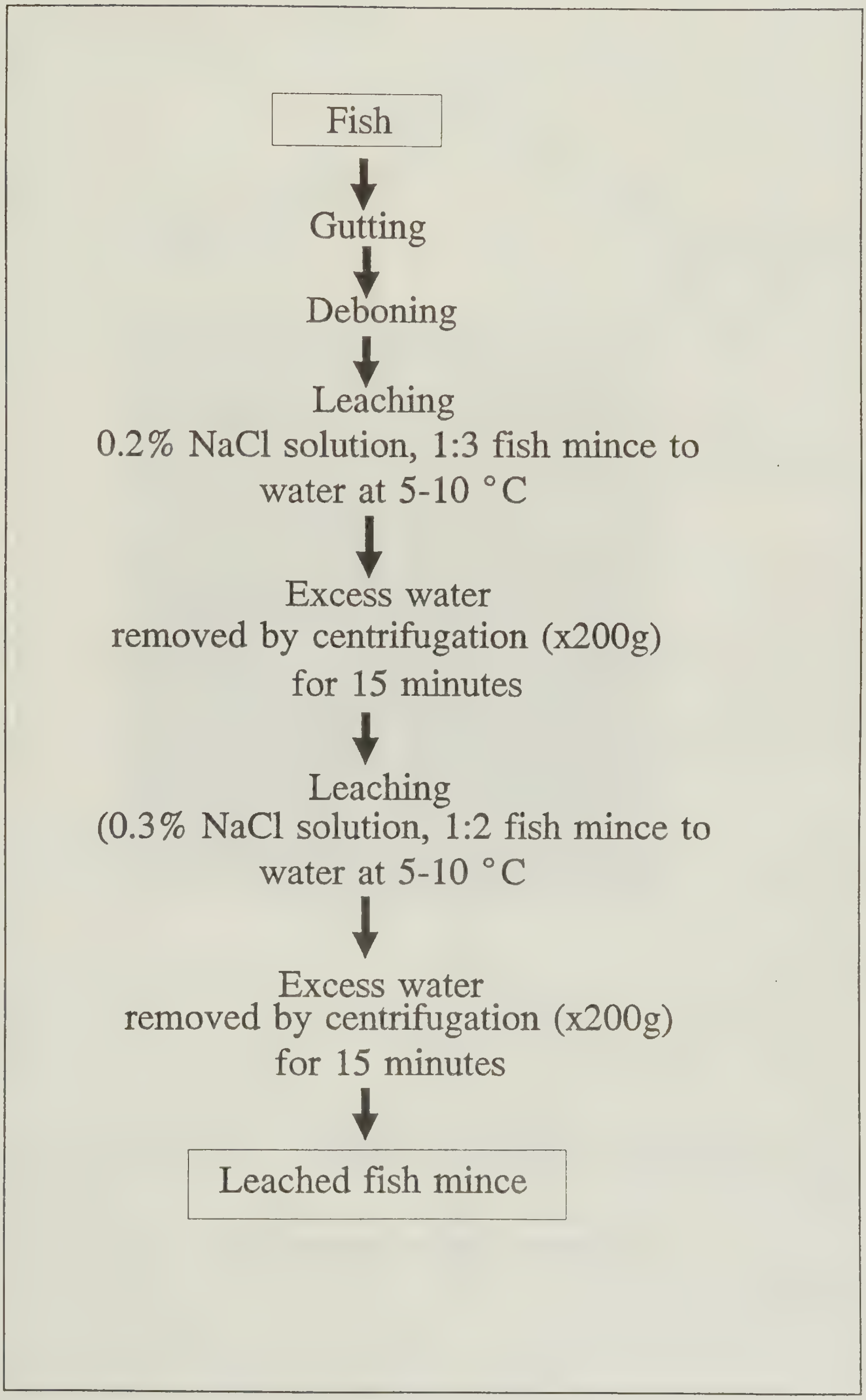
Fish
Gutting
Deboning
Leaching
0.2% NaCl solution, 1:3 fish mince to water at 5-10 °C
Excess water removed by centrifugation (x200g) for 15 minutes
Leaching
(0.3% NaCl solution, 1:2 fish mince to water at 5-10 °C
Excess water removed by centrifugation (x200g) for 15 minutes
Leached fish mince

Figure 1. Production of Leached Fish Mince

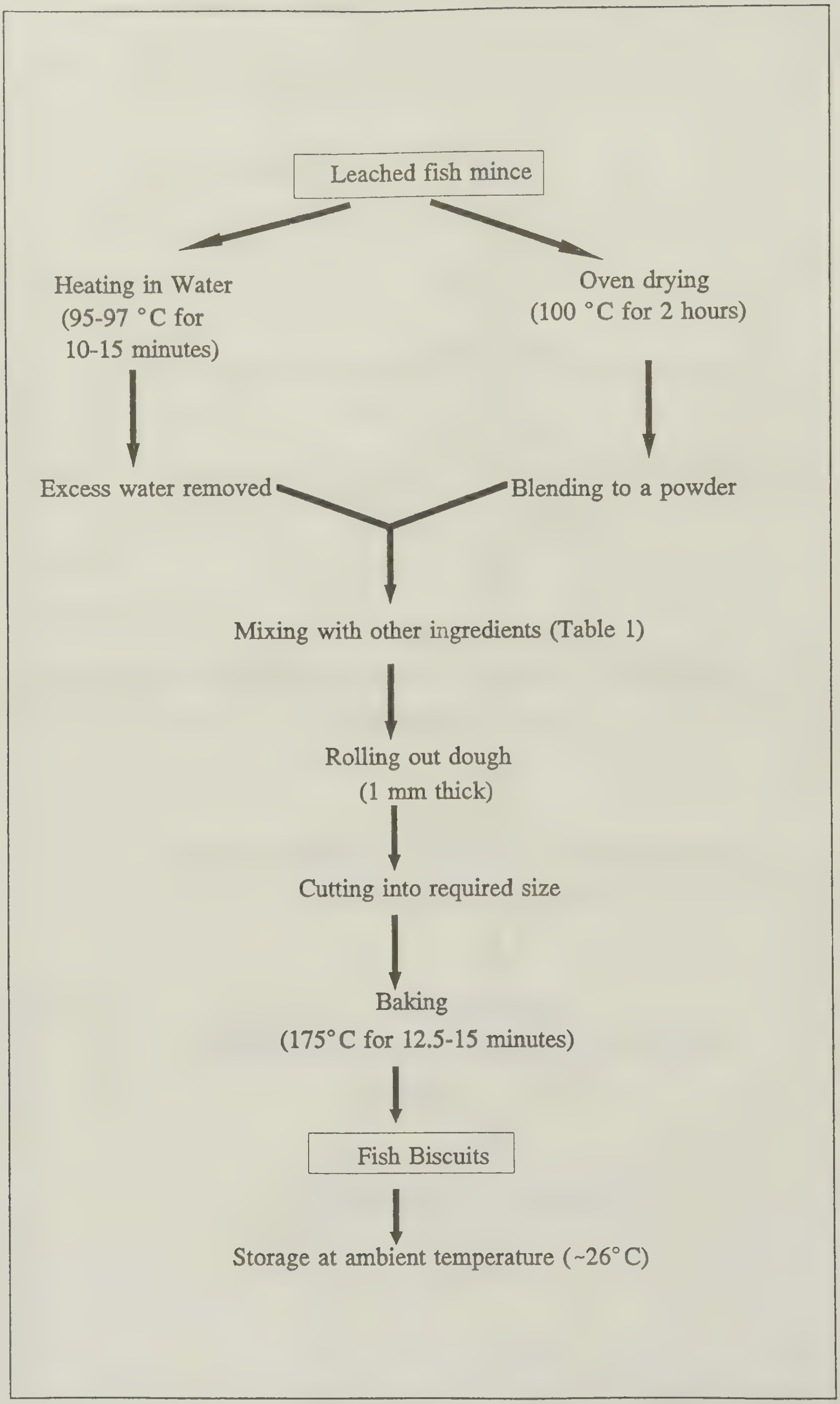
Leached fish mince
Heating in Water (95-97 °C for 10-15 minutes)
Oven drying (100 °C for 2 hours)
Excess water removed
Blending to a powder
Mixing with other ingredients (Table 1)
Rolling out dough (1 mm thick)
Cutting into required size
Baking (175° C for 12.5-15 minutes)
Fish Biscuits
Storage at ambient temperature (~26° C)

Figure 2. Production of Fish Biscuits

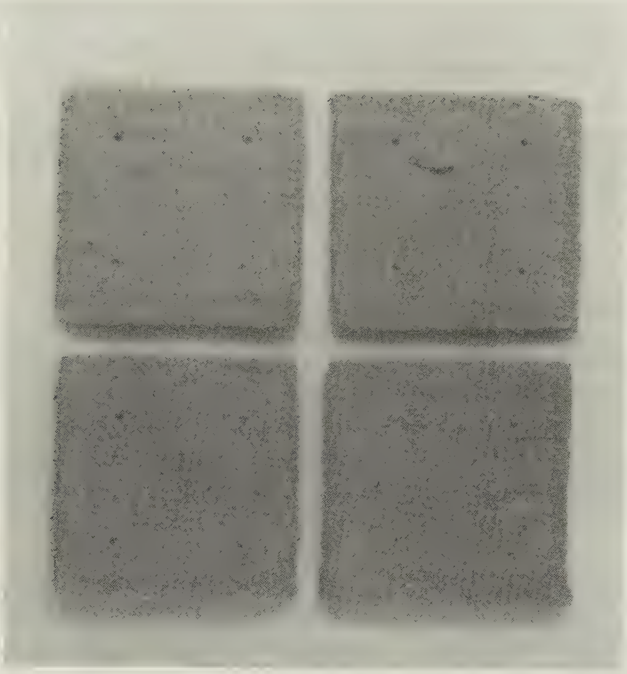

Figure 3. The Final Product - Fish Biscuit with 20g Water Heated Fish Mince

QUALITY EVALUATION AND STORAGE STABILITY OF BOILED DRIED ANCHOVIES

by

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ABSTRACT

Physical and chemical parameters for use as quality indices of boiled dried anchovies were identified. The effect of light, temperature and humidity on the quality changes were also determined. It was indicated that good quality boiled dried anchovies contained 8.0% salt, 19.9% ash and 43.3% moisture based on dry weight. Quality changes during storage under various conditions resulted in the increase in the TVB-N content, changes in colour and the development of lipid oxidation. Sensory evaluation showed that the quality of boiled dried anchovies can be maintained at an acceptable level for 3 weeks when kept at room temperature in the dark (27 ± 4°C, RH 73 ± 16%) and more than 6 weeks when kept refrigerated (7 ± 2°C, RH 80%).

INTRODUCTION

Boiled dried anchovies are traditionally produced in Southeast Asia from various species such as *Stolephorus* spp., *Anchoviella* spp. and *Engraulis*. spp. Currently the production method is based on simple technology. Freshly caught anchovies are usually processed on board. They are first boiled in 10% brine for 1-4 minutes using bamboo baskets with agitation during the cooking. The baskets are removed from the brine and allowed to drain and cool. On landing, the cooked fish are spread to dry in the sun usually for one fine day but for longer under poor drying conditions.

The packaging, handling and storage of boiled dried anchovies in the local market is either in large plastic bags and boxed in paper cartons for product from Malaysia, or in large polyethylene lined jute sacks or in bamboo woven baskets lined on the inside with plastic sheets and on the outside with jute cloth for product from Thailand and Indonesia, respectively.

The quality of the final product depends much on the skill of the processors during the cooking and drying processes, and also depends on the weather during drying. Local traders normally judge the quality of the products based on visual examination and experience for colour, smell and appearance of the products. The desired quality should be light cream or yellow colour with a silver strip along the body and head. Smell is also important and should be fresh smelling. The colour, a dry product and intact fish are the characteristics which command a higher price (SIRIM, 1984, MFRD, 1989).

Quality deterioration of boiled dried anchovies can occur rapidly in the hot, humid tropical region due to lipid oxidation causing the development of rancid odour and colour changes. An initial survey had indicated a wide range of quality of boiled dried anchovies is available in the local market. Therefore, it is necessary to identify suitable physical and chemical parameters for use as quality indices. In addition, a series of storage experiments were conducted to determine the effect of environmental conditions on the quality changes.

MATERIALS AND METHODS

Materials

Boiled dried anchovies were purchased from Tanjong Pinang, Pulau Bintan. After arriving at the PHT Laboratory, Marine Fisheries Research Department (Singapore), the fish were packed in KOP/ONY (PVDC-coated oriented polypropylene-oriented nylon) bags, vacuum sealed and frozen at -60°C.

Methods

The materials were thawed overnight at room temperature, packed in double-layered brown bags and stored at different conditions as follows:-

1. Refrigerator at 7±2°C and 80% RH.
2. Room temperature in a food cabinet with air circulation under normal light.
3. Room temperature in the dark by keeping in cardboard boxes.

The temperature and humidity in the room were continuously measured by using a recording thermohydrograph (ISUZU Electronic Precision Model 3-1124). The average temperature and humidity for the whole period of storage were 27±4°C and 73±16% respectively.

Quality assessment

The initial quality of the sample was assessed for moisture, lipid, protein, salt and ash using the method detailed in Hasegawa (1987) and acid insoluble ash by the method described in Malaysian Standard (SIRIM, 1984).

At weekly intervals during storage, the samples were monitored for quality changes including thiobarbituric acid number (TBA), total volatile basic nitrogen (TVB-N), moisture, and colour (L, a, b value) by Minolta Colour Meter (Model R-200). Percent breakage is determined by the amount of non-intact fish in 50 g of sample.

For sensory evaluation, the samples were packed in small plastic containers and assessed for colour, odour, texture, presence of mould and overall acceptability using 10 untrained panellists.

RESULTS AND DISCUSSIONS

The chemical composition of anchovies (Table 1) indicated that the lipid and protein content of boiled dried anchovies decreased due to the loss of such constituents in boiling water which was discarded after boiling. However, the salt content increased as salt was added to the boiling water.

Quality changes of boiled dried anchovies during storage under various conditions were determined. Lipid deterioration occurred as the TBA number of stored samples (Figure 1) decreased after two weeks storage under different conditions. Then they increased for samples stored in a refrigerator and at room temperature under dark condition whereas it decreased at room temperature when stored under light.

The result of TVB-N (Figure 2) showed slight increases in the first three weeks of storage at room temperature both in light and dark conditions, and then rapidly increased after three weeks of storage at room temperature under dark condition and four weeks at room temperature under light, which showed the results related to the sensory evaluations. The TVB-N content sample stored in a refrigerator showed an almost consistent result during five weeks storage.

The results of moisture content of boiled dried anchovies during storage under various conditions (Figure 3) showed that there was an increase in samples stored in the refrigerator at 7±2°C and 80% RH up to four weeks storage whereas they decreased after one week storage at room temperature in both light and dark conditions.

The colour of samples during storage changed from initial white/yellow to slightly yellow for the sample stored in a refrigerator, and changed to more yellow in the case of room temperature storage under both light and dark conditions (Table 2) which can be seen from the changes in b value indicating yellowish colour.

The quality of dried anchovies commanding a higher price are characterised by higher numbers of intact fish. Therefore, percentage breakage of the product was determined during storage. Due to improper handling and packing methods the higher percentage breakage was obtained from initial sample and slight changes during storage (Table 3). According to Malaysian Standard (MS 898) the A Grade dried anchovies should contain less than 5% breakage whereas B and C grade should contain 5-10% and above, 10% breakage, respectively.

Sensory evaluation (Table 4) showed slight changes in quality of the sample stored in the refrigerator at 7 ± 2°C, the odour remained fresh, the colour was white/yellow and there was a slight change in texture from slightly moist and soft to slightly damp but the overall quality was acceptable after 6 weeks storage. At room temperature under light the odour changes from fresh to mouldy, colour changed from slightly yellow to yellow especially on the belly of the fish. The overall acceptabilit,' was borderline at 4 weeks storage and terminated after 5 weeks due to high presence of mould. At room temperature under dark conditions, the sample showed changes in odour from fresh to mouldy after 3 weeks storage therefore the overall acceptability was terminated.

CONCLUSION

The chemical composition of boiled dried anchovies considered as good quality products consisted of 8.0% salt, 19.9% ash, 0.4% acid insoluble ash and 43.3% moisture content based on dry weight. The deterioration of samples during storage under various conditions resulted in the increase of TVB-N content, changes of colour and development of lipid oxidation. The quality of boiled dried anchovies can be maintained at an acceptable level for 3 weeks when kept at room temperature under dark conditions (27 ± 4°C, 72 ± 16% RH), 4 weeks at room temperature under light (27 ± 4°C, 72 ± 16% RH) and more than 6 weeks when kept refrigerated (7 ± 2°C, 80% RH).

Further study is required to assess additional qualities of the product e.g. acid value from lipid hydrolysis due to bacterial and fungal enzymes and available lysine to reflect the nutritional quality of the product.

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Table 1 Chemical composition of anchovies

Chemical parameters	Fresh anchovies	Boiled anchovies	Boiled, dried anchovies
Moisture (% dry wt)	552.0 ± 2.5*	185.1 ± 1.3	43.3 ± 0.1
Salt (% dry wt)	2.7 ± 0.2	13.4 ± 0.3	8.0 ± 0.3
Ash (% dry wt)	13.2 ± 0.5	ND**	19.9 ± 0.3
Lipid (% dry wt)	7.6 ± 0.4	6.3 ± 0.1	6.4 ± 0.4
Protein (% dry wt)	85.7 ± 0.7	72.3 ± 0.9	76.9 ± 0.6

* Mean ± Standard Deviation of three analyses
** ND = not done

Table 2 Colour (L, a, b) values of boiled dried anchovies during storage under various conditions

Storage condition	Storage time (weeks)							
		0	1	2	3	4	5	6
Refrigerated (7 ± 2°C)	L	65.5* ±2.6	66.2 ±0.4	66.8 ±0.7	65.7 ±1.4	65.6 ±0.3	65.0 ±0.6	63.5 ±1.1
	a	-0.6 ±0.0	-0.6 ±0.0	-0.7 ±0.0	-0.7 ±0.1	-0.8 ±0.0	-0.5 ±0.1	-0.6 ±0.0
	b	+14.3 ±0.9	+12.8 ±0.4	+12.8 ±0.5	+14.3 ±0.5	+13.6 ±0.9	+13.9 ±0.4	+12.6 ±0.1
Room temperature/ light (27 ± 4°C)	L	65.6 ±2.6	68.3 ±0.3	65.6 ±0.0	68.1 ±0.3	63.9 ±0.5	63.9 ±2.8	D**
	a	-0.6 ±0.0	-0.5 ±0.1	-0.4 ±0.1	-0.4 ±0.0	-0.1 ±0.2	-0.2 ±0.6	D
	b	14.3 ±0.9	14.9 ±0.0	16.1 ±0.2	17.2 ±0.3	17.6 ±0.4	18.6 ±0.1	D
Room temperature/dark (27 ± 4°C)	L	65.6 ±2.6	66.2 ±0.1	64.7 ±0.7	66.6 ±0.3	64.8 ±0.6	D	D
	a	-0.5 ±0.0	-0.5 ±0.2	-0.4 ±0.2	-0.4 ±0.0	-0.2 ±0.1	D	D
	b	+14.3 ±0.9	+14.8 ±0.0	+14.7 ±0.5	+16.2 ±0.7	+17.2 ±0.4	D	D

* Means ± Standard Deviation
** D = Discarded due to mould growth

Table 3 Percentage breakage (%) of boiled dried anchovies during storage under various conditions

Storage condition	Storage time (weeks)						
	0	1	2	3	4	5	6
Refrigerated (7 ± 2°C)	34.5 ± 7.1	34.1 ± 2.1	30.6 ± 3.2	35.0 ± 8.4	35.8 ± 8.0	38.8 ± 6.8	35.9 ± 12.1
Room temperature/ light (27 ± 4°C)	34.5 ± 7.1	47.9 ± 2.4	44.9 ± 3.1	54.5 ± 2.5	46.9 ± 0.7	34.2 ± 9.9	D*
Room temperature/ dark (27 ± 4°C)	34.5 ± 7.1	47.4 ± 2.1	60.3 ± 2.0	44.8 ± 4.5	45.0 ± 0.0	D	D

Table 4 Sensory evaluation of boiled, dried anchovies during storage under various conditions

Storage condition	Storage time (week)	Odour	Colour	Texture	Mould	Overall
Refrigerated (7 ± 2°C)	1	Fresh	White/yellow	Slightly moist & soft	Absent	Acceptable
	2	Fresh	White/yellow	V. slightly moist & soft	Absent	Acceptable
	3	Fresh	White/yellow	Pliable, moist & soft	Absent	Acceptable
	4	Fresh	White/yellow	Slightly damp	Absent	Acceptable
	5	Fresh	White/yellow	Slightly damp	Absent	Acceptable
	6	Fresh	White/yellow	Slightly damp	Absent	Acceptable
Room temperature/ light (27 ± 4°C)	1	Fresh	Slightly	Dry, firm	Absent	Acceptable
	2	Fresh	yellow	Dry, firm	Absent	Acceptable
	3	Stale	Yellow	Dry, firm	Absent	Acceptable
	4	Off-flavour	Yellow (belly)	Firm	Slightly present	Borderline
	5	Mouldy	Yellow (belly)	Firm	Highly present	Unacceptable
	6	-	Yellow	-	-	-
			-			
Room temperature/ dark (27 ± 4°C)	1	Fresh		Dry, firm	Absent	Acceptable
	2	Slightly	Yellow	Dry, firm	Absent	Acceptable
	3	rancid	Yellow	Dry, firm	Absent	Unacceptable
	4	Mouldy	Yellow (belly)	Firm	Highly present	Terminated
	5	Mouldy	Yellow	-	-	-
	6	-	-	-	-	-
		-	-			

IDENTIFICATION AND PURIFICATION OF PROTEOLYTIC ENZYMES FROM FISH PYLORIC CAECA

by

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ABSTRACT

The pyloric caecum is the part of the fish digestive tract which contains the proteolytic enzymes. In fish processing, pyloric caeca and other internal organs are thrown away as waste. The market potential for proteolytic enzymes as well as the high production of fish waste are the reason why experiments on using fish pyloric caeca as a source of proteolytic enzyme are important.

The experiment was done to identify the characteristics of enzymes produced from fish pyloric caeca. It was then followed by an enzyme purification process through several steps i.e. protein precipitation, fat removal, dialysis, freeze drying, and gel chromatography. The results revealed that the proteolytic enzyme produced, which was of tryptic type, had optimum activity at pH 8 and 45°C. Compared to commercial trypsin (MERCK), this tryptic type enzyme had a lower unit activity (U/ml/min), even though it had a higher specific activity (U/mg protein).

INTRODUCTION

Finfish consists of approximately 65% muscle, 2% liver, 8% gut (fore-gut, and hind-gut) and 25% of waste. The gut which consists of stomach, intestines, pyloric caeca, pancreas and other organs usually contain proteolytic enzymes.

The utilization of digestive organs as a source of proteolytic enzymes has been practised in some countries (Sukarsa, 1978). There is a wide variety of proteolytic enzymes present in the viscera and digestive tract of aquatic animals. Among these, trypsin, chymotrypsin, and pepsin are the most important. Pepsin is usually found in fish stomach and is the principal enzyme of the digestive juices, while trypsin is present in the pyloric caeca.

Trypsin is widely used in medical treatment, in beer and wine refining, or in meat tenderization. Bating, a part of leather tanning process, is usually carried out using trypsin, since this enzyme hydrolyses the elastin fibres in the skin.

The production of proteolytic enzymes from fish gut has good prospects in Indonesia because more than 50% of marine fish production, which is more than two million tons per year, is gutted before marketing as fresh (iced), frozen, or processed fish. However, some studies must be carried out especially on potential resources characteristics of enzymes, methods of extraction, and preparation for industrialization as well as on the application in industry.

Extraction is usually done by destruction of the cell wall of organs where the enzyme is located. This step can be carried out by homogenizing the organ (Whitaker, 1972). For this purpose, a potassium phosphate buffer solution, an ammonium sulphate solution, an aqueous salt solution, or distilled water can be used (Murachi *et al.* 1964, Purwanto, 1983). The most important factor in this stage of process is pH.

The crude enzyme extracted should be filtered or centrifuged to remove the remaining solid particles. The process subsequently involves the precipitation of the protein using inorganic salts, such as ammonium sulphate and sodium sulphate or organic solvents such as methanol and acetone (Stanbury and Whitaker, 1984), followed by filtration or by centrifugation to remove the lower molecular weight fractions. The isolated protein is then dialysed and concentrated either by vacuum-evaporation or ultrafiltration, followed by gel filtration and dehydration. Further purification steps can be by chromatographic separation of enzyme fractions, ion exchange absorption, electrophoresis separation, and crystallization of purified enzyme.

The catalyzing power of an enzyme is usually measured quantitatively by its activity on a substrate. The enzyme activity of most visceral and digestive tract enzymes from fish tend to be highest at neutral pH values. However, Hjelmeland and Raa (1980) found that the degradation rate of haemoglobin substrate was higher at pH 8 than at pH 7 and pH 6 when either crude enzyme extract or purified trypsin from capelin was applied. Besides the pH, season and feeding activity at the time of capture will also influence the enzyme activity. Nevertheless, the activity and thermal stability of fish enzymes varies from one species to another (Wheaton and Lawson, 1985). Generally, white fish have less proteolytic activity than do pelagic species (Mackie *et al.* 1971 in Wheaton and Lawson, 1985).

MATERIALS AND METHODS

The guts of grouper (*Epinephelus tauvina* F.) were collected from Pasar Senen, Jakarta, andch were iced and brought to the laboratory. The pyloric caeca were removed from the gut and stored in frozen condition.

The extraction was done by homogenizing the pyloric caeca in 0.05 M potassium phosphate (K_3PO_4) in the ratio of 1:4, followed by filtration to separate the solid particles.

The crude enzyme produced was then examined for its activities at various pH and temperatures and its thermal stability. Activity measurements were done at 37°C using Bergmeyer's method (Bergmeyer, 1983).

Purification of the enzyme was started by protein precipitation which was conducted by gradual addition of ammonium sulphate crystals while stirring until saturated. It was then stored overnight at 4°C and finally separated by centrifugation at 10°C 15,000 rpm. for 15 minutes.

Fat separation was done by mechanically shaking the precipitated protein in 10 ml of diethyl ether for each gram of enzyme for one hour. The dissolved fat was removed afterwards.

The following step of purification was the dilution in universal buffer at pH 8 before dialysing in a cellophane bag at 4°C for 48 hours. The dialysed enzyme was frozen at -4°C and freeze-dried before storage at 4°C for subsequent purification. The last step was fractionation using a Sephadex G-100 column. The fractions were then measured for their absorbency at 578 nm by adding 2 ml of 0.4 M sodium carbonate (Na_2CO_3) and 0.5 ml of Folin's reagent for each 0.5 ml of sample.

The activities of enzyme after each step of purification were measured at pH 8, 45°C using the method of Bergmeyer (Bergmeyer, 1983).

RESULT AND DISCUSSION

Optimum pH for activity

Test on casein substrate at 37°C and at pH 7.0; 7.5; 8.0; 8.5; 9.0 and 9.,5 showed that the enzyme activity tended to increase with increase of pH, and reached a maximum at pH 8, then decreased gradually at higher pH (Figure 1).

This result agrees with the result of Sukarsa's experiment (1978) reporting that the proteolytic enzyme extracted from the pyloric caeca of snakehead, snapper, mackerel, and mullet have an optimum activity at pH 8.6; 8.6-8.8; 8.8; and 8.8 respectively at 40°C.

Optimum temperature for activity

The result of experiments on the activity of the enzyme at 30, 37, 45, 55 and 65°C is shown in Figure 2. It can be seen that rise in temperature leads to an increase of enzyme activity. However, the activity decreased significantly at temperatures over 45°C, and this is probably due to heat denaturation of the enzyme.

Thermal stability

Thermal stability tests were done both at and above the optimum temperature. In this test, residual activity (the percentage of enzyme activity after incubation compared to its activity before incubation) were measured. The result showed that at the optimum temperature (45°C), the residual activity increased from 100% to 145.68% during the first 20 minutes, which presumably occurred due to the changes in tertiary structure. However, 20 minutes seemed to be the maximum time for the enzyme to remain stable, because during a longer period of incubation the residual activity decreased significantly and after 100 minutes of incubation, the residual activity was only about 50% (Figure 3).

In the second test, the enzyme was exposed to heat at 70, 80 and 90°C, and in boiling water (98°C) for 10 minutes. It was shown in Figure that at 70°C the residual activity was still about 50% which then drastically decreased with the increase of temperature, until finally no activity can be detected at 100°C.

Enzyme activity during the purification process

After each step of purification, measurement of unit activity, total activity, protein content by using the Lowry method, specific activity, and degree of purity were conducted. Unit activity is the activity per unit of material (U/ml or U/g), total activity is a multiplication of unit activity and quantity, specific activity is the activity per mg of protein, while degree of purity is the ratio between specific activity of a certain step and the specific activity at the previous step.

It was found that the specific activity and the degree of purity of the enzyme increased at a further step of purification (Table 1). The high concentration of ammonium sulphate salt will enhance the decrease of globular protein solubility. That is why this method of precipitation is usually called "salting out". Storing the solution overnight at 4°C also helps the aggregation of the protein molecules, and hence accelerates the precipitation of the protein.

The increase of enzyme specific activity after the removal of fat showed that the method of protein precipitation used is not complete enough to purify the enzyme, because it could not remove the fat, which could reduce enzyme activity.

Dialysis was carried out to separate the inorganic salt from the macromolecules of enzyme protein. This step is important in gel chromatography separation. This separation also requires small volume and high concentration of a sample. For this purpose, freeze drying is required, though this process has no effect in increasing enzyme activity.

Table 1 Characteristics of enzyme in the purification steps

Step of purification	Quantity	Unit Act.	Total Act.	Protein cont.	Specific Act.	Degree of purity
Raw material	320.85 g					
Crude extr.	1235.00 ml	1.976 U/ml/min	2440.360 U/min	10.067 mg/ml	0.196 U/mg prot/min	1.000
Precipitation	54.772 g	58.781 U/g/min	3219.553 U/min	100.871 mg/g	0.583 U/mg prot/min	2.975
Fat removal	36.372 g	150.630 U/g/min	5478.714 U/min	114.061 mg/g	1.321 U/mg prot/min	6.735
Dialysis	184.107 ml	26.506 U/ml/min	4879.940 U/min	10.820 mg/ml	2.450 U/mg prot/min	12.500
Freeze drying	5.971 g	442.980 U/g/min	2645.034 U/min	157.380 mg/g	2.815 U/mg prot/min	14.362
Gen Chr. Separation						
Fract.no.25	107.478 ml	1.290 U/ml/min	138.647 U/min	0.226 mg/ml	5.708 U/mg prot/min	29.122
Fract.no.26	107.478 ml	1.392 U/ml/min	149.609 U/min	0.248 mg/ml	5.613 U/mg prot/min	28.630
Fract.no.27	107.478 ml	1.420 U/ml/min	152.619 U/min	0.244 mg/ml	5.820 U/mg prot/min	29.694
Fract.no.35	107.478 ml	1.425 U/mo/min	153.156 U/min	0.116 mg/ml	12.284 U/mg prot/min	62.673

After gel chromatography separation it was shown that fraction no. 35 had the highest unit activity, specific activity and degree of purity, followed by fraction nos. 27, 26, and 25.

Comparison to other trypsin

Compared to commercial trypsin (MERCK), the crude enzyme extracted from the pyloric caeca of grouper had a lower unit activity, protein content, as well as specific activity (Table 2). However, after a series of purification steps, the specific activity was much higher than the commercial enzyme.

Table 2 Characteristics of several enzymes

Enzyme	Unit Activity	Protein content	Specific Activity
Experimental crude trypsin	1.976 U/ml/min	10.067 mg/ml	0.196 U/mg prot/min
Experimental purified trypsin	1.425 U/ml/min	0.115 mg/ml	12.284 U/mg prot/min
Commercial trypsin (MERCK)	64.660 U/g/min	39.628 mg/g	1.632 U/mg prot/min

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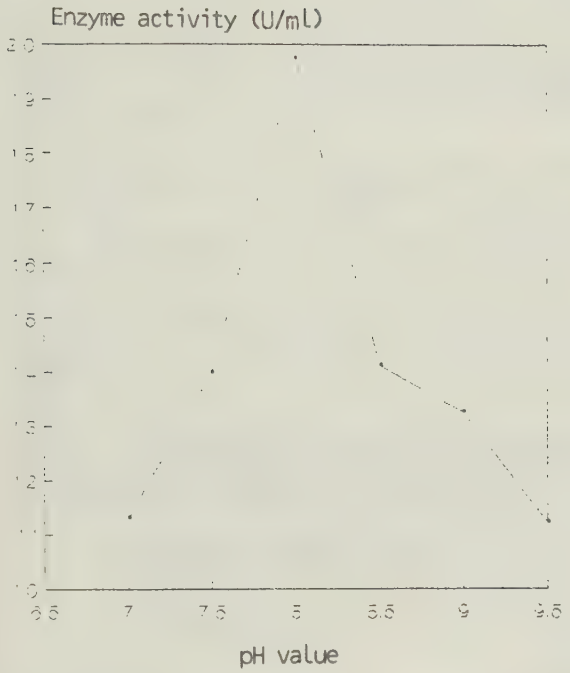

Fig. 1. Enzyme activity at various pH

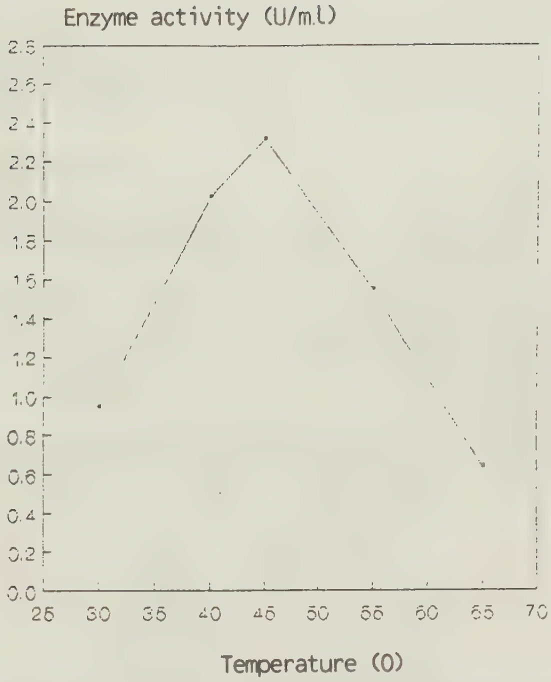

Fig. 2. Enzyme activity at various temperatures

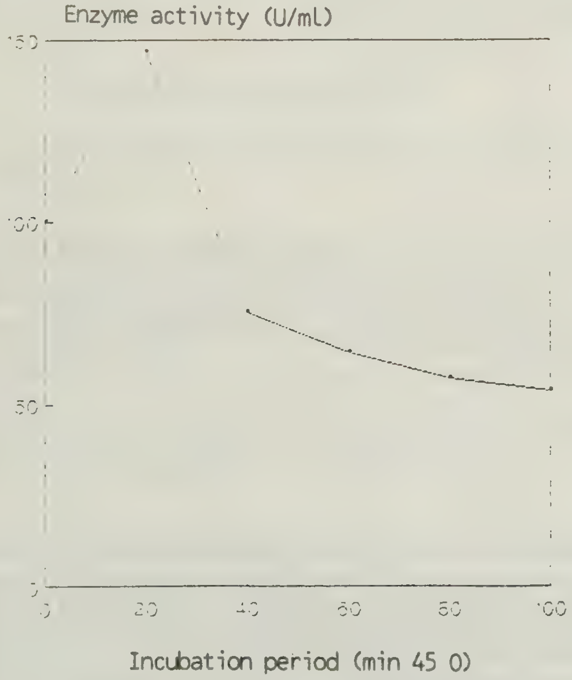

Fig. 3. Thermal stability of enzyme at its optimum temperature

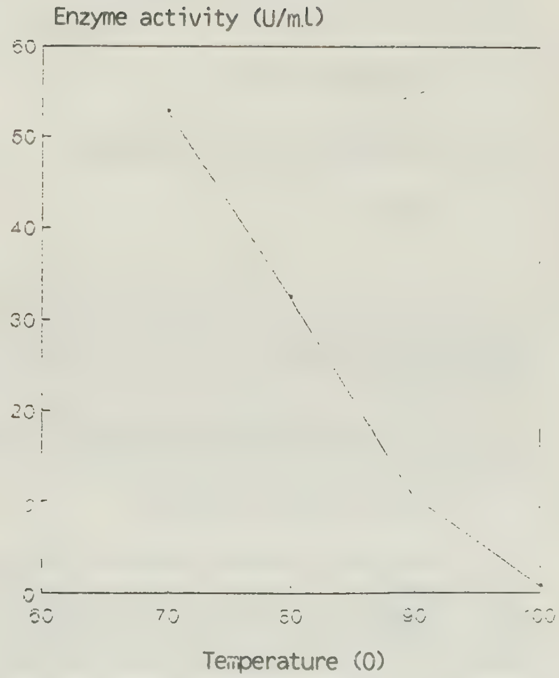

Fig. 4. Thermal stability of enzyme at various temperatures

EXTRACTION AND COMPOSITION OF DOGFISH LIVER OIL

by

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ABSTRACT

Oil was extracted from dogfish livers by the Bligh and Dyer, Soxhlet and steaming extraction methods. Oil yields using the steaming method were lower than from the solvent extraction methods (22 and 68% w/w). The vitamin A content of the oil produced by steaming was 54 mg retinol/100 g oil; slightly higher contents were recorded in the solvent extracted oils. It appears that Vitamin A is present in livers as the palmitate ester, because in unsaponified oils only one HPLC peak was observed, corresponding to a retinyl palmitate standard. Detailed chemical composition of the oils is described and discussed.

INTRODUCTION

Vitamin A deficiency is the second most common form of malnutrition. A potentially valuable source of Vitamin A is the livers of cartilaginous fish species (sharks, dogfish). In south and southeast Asia the total landings were about 160,000 tonnes. Assessment of the amount of oil extracted by various methods, the chemical composition of the oil and Vitamin A content are essential in order to evaluate the use of this potential source of Vitamin A. This work investigated these factors using livers of dogfish (*Squalus acanthias*) since shark livers were not readily available.

EXPERIMENTAL

MATERIAL AND EQUIPMENT

Livers

Livers from dogfish (*Squalus acanthias*) which had been caught in the North Sea between May and August 1985 were obtained from Grimsby fish dock. The livers were chilled in ice prior to arrival in the laboratory. They were in good condition and did not show any visual trace of spoilage. After being chopped and mixed, they were stored at -60°C until required.

EXTRACTION METHOD FOR DOGFISH LIVER OIL

Direct steaming method

After being thawed at ambient temperature, the chopped livers were weighed (500-1000g), placed in a muslin bag and put on a metal screen over an aluminium pan. The pan containing the liver was cooked with steam in a retort at 70-80°C for 30 minutes. The liver in the muslin bag was then hand-pressed and the oil-water released was combined with the oil-water released from the liver during steaming. The oil was separated from the water by centrifuging at 2000 rpm for 15 minutes, dried with anhydrous sodium sulphate and separated from the dried material by filtering under suction using a Buchner funnel.

Solvent (Soxhlet) method

After thawing at ambient temperatures the liver (300g) was weighed and mixed with anhydrous sodium sulphate (1:1). The mixture was placed in thimbles in a large Soxhlet extractor and extracted continuously with 1500 ml of petroleum ether 40-60 for five hours. The solvent containing the extracted oil was evaporated under vacuum on a rotary evaporator and the oil fraction was shaken under high vacuum to remove the last traces of petroleum ether with protection from the light by wrapping the flask with aluminium foil.

Bligh and Dyer method

After being thawed at ambient temperature, the liver (100g) was weighed into a 2 1 beaker. To this, distilled water (100 ml) was added and mixed. Chloroform (200 ml) and methanol (400 ml) were added and the mixture was homogenised for two minutes whilst being cooled in ice. More chloroform (200 ml) was added and homogenised for one minute, followed by the addition of distilled water (200 ml), and finally homogenised for 30 seconds. The mixture was then centrifuged at 2000 rpm for 20 minutes. The aqueous layer was removed by suction. The chloroform fraction was dried with anhydrous sodium sulphate, filtered and the chloroform evaporated under vacuum on a rotary evaporator. The oil fraction remaining was shaken under high vacuum to remove the last traces of chloroform with the flask being wrapped in aluminium foil to exclude light.

ANALYTICAL PROCEDURES

Free fatty acid (FFA)

To a mixture of diethyl ether (25 ml) and absolute ethanol (25 ml), 1% phenolphthalein (1 ml) was added and the mixture carefully neutralised with 0.100 M sodium hydroxide. The lipid sample (approx. 1g) was dissolved in the neutralised solvent and titrated with the sodium hydroxide (with shaking) until a pink colour persisted for 15 seconds. Free fatty acid was calculated as percent oleic acid.

Peroxide value (PV)

The method used was adapted from that of Wood and Aurand (1977).

Iodine value

The iodine value was analyzed by Wij's method (Pearson 1981).

Thiobarbituric acid value (TBA)

The thiobarbituric acid value was analyzed using the method of Sinuhuber and Yu (1973) with slight modifications.

Fatty Acid Methyl Ester (FAME)

Methyl esters were prepared based on the method of the British Standard of Analysis of Fats and Fatty Oils (1980) with slight modification. These were analyzed using a polar packed column, active phase SP2330. Nitrogen gas was used as carrier with flow rate 20-23 ml per minute. The temperature programme used was 180°C for the first four minutes, then increasing at 5°C per minute to 230°C. A flame ionizing detector was used with the signal automatically presented as percentage of the total peak area (C14 upwards).

Vitamin A analysis

Quantitative analysis of Vitamin A was done in two steps namely extraction and separation. The extraction of retinol was carried out using a modification of the procedure in the methods given in Anon (1966).

Retinol in the unsaponified matter was then analyzed by HPLC using 25 cm of C18 column, eluted with 90% methanol. Qualitative analysis of Vitamin A was done by injecting the oil, diluted in chloroform directly into HPLC using a C18 column with dry hexane/wet hexane/dietyl ether/isopropanol (47/47/5/1) as eluent and UV at 325 nm as the detector.

RESULT AND DISCUSSION

Oil Yield

The oil yields from the Bligh and Dyer and Soxhlet extraction methods were similar and higher than for the steaming method because steaming results in the thermal rupture of the liver cells and so releases the oil, but it may be that oil which is more closely held by the proteinaceous liver tissue is not released under these conditions, Bailey (1942) found that after decanting the first portion of free oil from the steamed and centrifuged dogfish liver, the residue gave an oil yield from 2 to 10% of the total oil when it was solvent extracted.

The Bligh and Dyer and Soxhlet extraction gave results in agreement with data for the oil content of dogfish liver oil caught from many areas of the Pacific and Japan which have been cited as 50-70% (Brody, 1965; Kayama *et al*, 1969; Kizevetter, 1973; Murray, 1983).

The yields in experiments 4 and 5 were lower than experiments 1, 2 and 3, and this may be due to the loss of oil during freezing and thawing many times, as mentioned by Brody (1965), or loss of free oil as mentioned by Bailey (1942) who reported only very small quantities of Vitamin A in this free oil.

Chemical and nutritional composition of extracted oil

The chemical composition of the oil produced by the three different methods of extraction are tabulated in Table 2.

The quality of the dogfish liver was good with no visual or olfactory evidence of spoilage, and this was indicated by the low free fatty acid (0.24-0.47), since spoiled liver contains a high level of free fatty acids (Shenoy and Dey, 1984).

From Table 2 it can be seen that the rancidity parameters (PV and TBA values) of the oil produced by these three different methods were low (PV of 0.39-0.84 and TBA of 1.20-1.80). However, the PV and TBA values for the oil produced by the steaming method were slightly lower than for the oils extracted by the Bligh and Dyer and Soxhlet extraction methods. This may indicate that during extraction by the Bligh and Dyer and Soxhlet methods some slight deterioration may occur and this may be caused by the chemicals used as solvents (chloroform, methanol, petroleum ether). Bolomey (1946) suggested that the solvent may contain peroxides.

Table 1 % Oil Yield (w/w) from different methods of extraction of dogfish liver

Experiment	Bligh & Dyer	Soxhlet extraction	Steaming method
1	69.7	69.6	23.0
2	69.6	69.9	23.5
3	69.2	-	24.8
4	62.0	61.0	20.0
5	62.8	61.8	19.0
Mean value	66.7	65.6	22.1
% C.V.	5.2	6.4	10.0

Note: Experiments 1, 2 and 3 were carried out using fresh liver. Experiments 4 and 5 were carried out after the livers had been stored at -60°C for 5-9 months. All oils were a pale yellow colour, but the oils extracted by steaming were always a lighter colour.

Table 2 Chemical Composition of Extracted Dogfish Liver Soils

Analysis[1]		Bligh and Dyer Extraction	Soxhlet Extraction	Steaming Extraction
Free fatty acid		0.41	0.47	0.24
(as % of oleic acid)		(4.8)	(6.3)	(4.2)
Peroxide Value		0.84	0.66	0.39
(meq/kg oil)		(7.1)	(3.0)	(2.6)
TBA Value		1.70	1.80	1.20
(mg M.A/kg oil)		(5.9)	(5.6)	(0.0)
Iodine value		117.7	109.4	132.7
(g of iodine/100g oil)		(1.9)	(0.9)	(0.1)
Saponification value		310.4[3]	122.7	138.7
(mg KOH/g oil)		(1.5)	(1.1)	(2.7)
Phospholipid		12.21	4.23	1.26
(%)		(3.2)	(2.4)	(0.0)
Unsaponifiable matter		17.33	16.44	16.04
(%)		(2.2)	(0.5)	(1.3)
Vitamin A[2]		61.86	66.82	54.56
(mg retinol/100g oil)		(4.7)	(2.6)	(1.0)
Vitamin E		27.47	25.55	24.26
(mg retinol/100g oil)		(1.6)	(9.8)	(14.3)
Squalene		28.31	23.17	40.1
(mg/100g oil)		(3.2)	(4.1)	(2.9)
Cholesterol		1.15	1.14	1.13
(g/100g oil)		(5.4)	(4.9)	(3.7)
Fatty acid profile	C14:0	1.0-2.0	2.1-2.2	2.1
(percentage composition)				
	C16:0	13.3-15.4	15.8-15.9	15.1-13.3
	C16:1	5.4-5.5	6.2-6.7	6.5-6.6
	C18:1	23.7-24.0	27.3-27.7	27.5-27.9
	C18:2	1.9-2.0	0.2-0.6	0.2-0.3
	C20:1	12.1-12.5	12.5-12.8	12.7-12.8
	C20:5	4.4-4.6	4.5-4.8	4.5-4.8
	C22:1	14.7-15.3	14.7-15.1	14.5-14.6
	C22:5	2.6-2.8	2.3-2.4	2.4-2.6
	C22:6	11.1-11.7	10.0-10.5	9.9-10.2

[1] Values are means of triplicate determination; percentage coefficients of variation are given in parenthesis; ranges only are shown for fatty acid composition (triplicate determination).

[2] HPLC analysis.

[3] See text.

The iodine value of the oil produced by the steaming method was higher (133) than for the other two methods (109-117). Thus the oil produced by the steaming method has a higher average level of unsaturation.

Squalene, a long chain hydrocarbon with six double bonds has been found in the liver of many species of shark and is responsible for the high iodine values observed (Kizevetter, 1973). The iodine value of squalene is calculated to be 371 and this was found experimentally (364.8-372.1g iodine/100g). From Table 2 it can be seen that the squalene content of oil extracted by the steaming method, thus the squalene content is not responsible for the high PV. Carotenoids, which have more double bonds per molecule, may be responsible for the higher PV.

It was found that the saponification values of oil extracted by the Bligh and Dyer method (310) was much higher than the others (123-139). This corresponds with the higher phospholipid content (12.2%).

The different phospholipid content for the Bligh and Dyer (12.2%) and for the solvent method (4.2%) are due to the different polarities of the solvent used - the Bligh and Dyer uses methanol-chloroform which is more polar than the petroleum ether used in the Soxhlet method.

The important factor in the Soxhlet method is not only the ratio of solvent volume to the material weight, but also the frequency of extraction. Thus in this experiment, the different polarity of solvent is the most important factor in the different phospholipid content of oil extracted by the Bligh and Dyer and by the Soxhlet method. The phospholipid content for oil produced by the steaming method is lowest (1.31%) and indicates that steaming cannot release the phospholipid which is usually present in the cell membranes.

The vitamin A content, measured as retinol, of the three differently extracted oils, was slightly different, with the oil produced by the solvent extraction method having the highest concentration (Table 2). This difference did not correspond with the amount of oil extracted (Table 1) and therefore was not caused by a dilution factor but more likely by a solubility factor in that Vitamin A is readily soluble in the non-polar solvent used. Thus the use of solvent increased both the amount of oil extracted and the concentration of Vitamin A in the extracted oil. HPLC of the oil without saponification showed only one peak with a retention time the same as that of retinyl palmitate. This result agrees with many reports that Vitamin A in many biological sources, including fish, is in the ester form. Using reverse phase HPLC for determination of retinol after saponification, two peaks were observed - trans-retinol and cis-retinol. The formation of cis-retinol (8% to 15% of total retinol) can result from isomerisation during sample preparation, especially during saponification, indicating that by far the major form occurring in the unsaponifiable matter of dogfish liver oil is the trans form. Anon (1986) stated that fish oil and concentrate may contain 30-40% cis-Vitamin A of the total Vitamin A and also Sevel *et al.* (1984) reported that fish liver oil may contain 35% cis-retinol. This high figure was not found in the present work. These apparent lower values are probably due to the lower extinction coefficient of the cis-isomer. Rao (1967) reported that poly-cis lyopenes have extinction coefficients which are about half that of the all-trans form.

The Vitamin A range of 54 to 67 mg/100g oil (1 I.U. = 0.3 ug of retinol /100g oil). This figure is within the range of the Vitamin A contents of shark liver oil reported by many authors, i.e. 160-360,000 I.U./g oil, (Brody, 1965; Kizevetter, 1963; Murray, 1983). The very wide range in Vitamin A content in many shark liver oils is related to the season, species, size of fish and location where the fish were caught. The other important factor is method of analysis, especially when the Vitamin A is analyzed by the Carr-Price method. Different methods of analysis have been used (e.g. Carr-Price method with or without modification, colorimetry based on the reaction with trichloroacetic acid, spectrofluorometry, UV absorbtion, HPLC), and can give different apparent values for Vitamin A content.

It was found that the Vitamin E content in the oil was between 20-30 mg/100 g oil, and was similar for all the differently extracted oils. Although the coefficient of variation was found to be high, especially for oil extracted by the steaming method (14.3%), these oils contain Vitamin A in similar amounts to the 26 mg/100g oil reported of cod liver oil by Sonntag (1979). However, no report of Vitamin E content in shark liver oil could be found in the literature.

Cholesterol was found in similar amounts (1.1%) in the three differently extracted oils, and these results agree with those reported by Kayama *et al.* (1969) that liver oil produced from dogfish landed in Japan contain 1.03% of sterols which is 90% cholesterol. In contrast to the varying squalene content of shark liver oils, sterol or cholesterol contents in six species of shark (including dogfish) were no different (Kayama *et al.* 1969). Iwasaki and Harada (1984) reported that using GC analysis, blue shark liver tissue contains 1.16% sterol (which was 96% cholesterol). Although these figures were obtained after saponification of freeze-dried liver tissue, they are similar to those previously quoted for oils because blue shark usually contains high amounts of oil in the liver tissue.

From Table 2 it can be seen that the fatty acid profiles were similar for the differently extracted oils, despite differences in phospholipid contents.

As for most fish oils, the dogfish liver oil contained reasonable levels of C22:5 and C22:6 (about 2.5 and 10.5% respectively) which are nutritionally important in the human diet. However, C10:1 and C22:1 were found in higher concentration (12.5% and 14.5% respectively), which is similar to those found in many species of shark liver or body oil (Peyronel *et al.* 1984; Ackman, 1982 and Gruger *et al*, 1964). It is still under discussion whether erucic acid (C22:1 W-9) causes cardiac lipidosis, but Ackman (1982) reported that C22:1 W-9 (erucic acid) is not present to any important degree (6.5% of total C22:1), but rather the dominant isomer is the N-11 in fish oils.

CONCLUSIONS

The oil extracted by the steaming method gave a lower oil yield (22%) compared with the chemical methods (± 66%). The steaming method also gave lower Vitamin A contents (54.56 mg%) compared with the chemical methods (more than 60%). The other chemical parameters were not very different except for phospholipid content. However, the steaming method is applicable in practice because the method is easier, cheaper, quicker, and can probably be applied in rural areas.

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THE UTILIZATION OF SHRIMP HEAD WASTE AS SHRIMP HEAD MEAL

by

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ABSTRACT

The utilization of shrimp head waste as shrimp head meal was investigated. The results show that the processing gave a yield of 20 percent. Good quality (*Penaeus monodon)*. Proximate composition was: 7.88%, 22,03%, 8.02%, 45.40 and 0.82 moisture, total ash, fat crude protein and cholesterol respectively. The technology of processing was found to be simple and inexpensive.

INTRODUCTION

The production of frozen shrimp for export in 1990 was 80 883 t and is predicted to increase. From this total production 90% percent was exported as headless frozen shrimp. The yield of frozen headless shrimp is 70% so the head waste is about 21,800 t/year. Shrimp heads have been a serious disposal problem in some cities in Indonesia.

Some investigations on the utilization of shrimp head waste had been carried out, and use for animal feed seems to be a solution.

MATERIALS AND METHOD

Materials and equipment

The equipment used was as follows:

- plastic trays
- cooker
- boiling drum
- drying rack
- milling machine

Procedures

The shrimp heads from the frozen shrimp industry was washed free from dirt in water with stirring. The cleaned heads were then steamed for 30 minutes. After steaming, the shrimp head was dried on a rack under the sun for about 15 h turning regularly until dry. After drying, the dry shrimp head was milled and sieved to mesh size according to requirement. The shrimp head meal was then packed in polyethylene sacks.

RESULTS AND DISCUSSIONS

The yield at the end product of shrimp head meal from shrimp head waste was 15 percent. The technology of processing was found to be very simple with inexpensive equipment except for the milling machine. However, the milling step could be done without the milling machine namely by crushing the dry shrimp head and sieving to a mesh size as required.

Chemical analysis for the shrimp head meal was carried out to determine the quality of the end product compared to other meal as a raw for animal feed. The proximate analysis gave results as shown in Table 1 below.

Table 1 Proximate composition of shrimp head meal of *P. monodon*

Parameter	(%)
- moisture	7.88
- total ash	22.03
- fat	8.02
- crude protein	45.40
- cholesterol	0.88

From Table 1 it can be seen that crude protein content was 45.40%. Crude protein was analyzed by the Kjeldhal method which determined all the nitrogen present in the sample. Shrimp shell also contains other important N compounds such as chitin. When chitin was analyzed, the result was approximately 2.5% nitrogen. It is assumed that N total is N-protein plus N-chitin, so true protein content was approximately 27%. However, compared to other sources of animal feed raw material, as shown in Table 2, the shrimp head meal appeared to have a high content of crude protein (45.4%), even higher than the crude protein content of dried shrimp (Tables 1 and 2).

Table 2 Proximate composition of some meals as raw material of shrimp feed

Products	Protein (%)	Fat (%)	Moisture (%)	Ash (%)
- dried shrimp	43.55	4.60	10.15	32.48
- imported shrimp meal	39.88	4.55	10.53	36.01
- soy meal*	43.99	2.41	11.22	6.73
- corn meal*	7.63	4.43	11.01	2.70
- imported fish meal*	59.85	4.28	9.61	24.87

* Source: BBAP, 1986

Table 1 also shows that shrimp head meal contains 0.88% cholesterol (HPLC analysis). This could be important in this meal as raw material for shrimp feed. The other advantage of shrimp head meal as a raw material of shrimp feed is that it contains carotenoids. Both cholesterol and carotenoid (astaxanthin) are essential for the growth of cultured shrimp.

These experiments indicate that the technology of utilization of head shrimp waste as shrimp head meal is simple and inexpensive, and could give economic benefit and solve the problem of disposal.

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THE UTILIZATION OF SEAWEED AS SHEET AGAR

by

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ABSTRACT

The utilization of the red seaweed *Pterocladia sp* as sheet agar was investigated. The yield as agar was 20-25 percent from dried raw material (40% moisture), consisting of grade 1 (67-78%), grade 2 (5-15%), and grade 3 (12-26%). The proximate composition was 15-20% moisture; 20-30% total ash; 1-3% protein and 1% acid insoluble ash. The gel strength of 2% agar solution was 150-400 g/cm^2.

INTRODUCTION

The production of seaweed in Indonesia increased rapidly after the introduction of seaweed culture. However, the technology of processing is very limited, therefore most seeweed is exported as dry raw material whereas the import of agar increases annually.

Sheet agar is a product which has many advantages in a developing country like Indonesia. It has simple methods of processing, inexpensive equipment and easy handling of end products. Experiments in producing sheet agar have been carried out, and the results are discussed below.

MATERIAL AND METHODS

Materials

The seaweed used in this experiment was red seaweed (*Pterocladia sp*) characterized by red to black colour, brittle and long thallus. The calcium and potassium hydroxides used were analytical grade.

Equipment

The following main pieces of equipment were used in the experimental work:

Box for washing and soaking.
Boiling drum.
Filter.
Cutter.
Press.
Drying equipment.

Processing

Cleaning: The raw material (dried seaweed) was submerged in water for two hours, then washed with stirring. Dirt was removed and the seaweed washed again until clean.

Paling: The cleaned seaweed was soaked in water with 0.5% calcium hydroxide for 5-10 minutes, then washed with water. The cleaned seaweed was then dried under the sun with regular turning until dry. The seaweed was then soaked in water overnight, then washed again several times with squeezing until free from calcium hydroxide.

Extraction: A two step extraction was applied. First, the seaweed was extracted in water with a volume of 14 times the weight of the dried raw material for 2 hours at a temperature of 85-95°C. The mass was then filtered and re-extracted with 6 volumes of water per weight of raw material for 2 hours at a temperature of 85-89°C, then filtered again. The filtrates from the first and second extractions were combined for setting into a gel.

Setting into a gel: The combined filtrate was then mixed with 2-3% potassium hydroxide and heated with stirring continuously for 15 minutes. After heating, the filtrate was poured into a mould and left overnight to set into a gel.

Cutting, pressing and drying: The gel formed was then sliced to a thickness of 8-10 mm. The pieces were wrapped in a cotton cloth and pressed gradually. After pressing, the agar in the wrap was dried until very dry under the sun. The dried produce was sorted according to quality.

RESULTS AND DISCUSSION

Yield of the end product

The yield of sheet agar from dried seaweed (40% moisture) was 20-25%, of which 67-68% was first grade, 5-15% second grade, and 12-26% third grade. The quality grade was determined according to the following characteristics:

Grade 1: White and clean, thin and unfragile.
Grade 2: White to yellow, thin enough, a bit dull, limited dirt.
Grade 3: Yellow to brown, thick and brittle, widespread dirt.

Proximate composition and gel strength

The proximate composition of the sheet agar was as follows: Moisture content (15-20%), total ash (20-30%), protein (1-3%), and acid insoluble ash (1%). The gel strength was measured by texture analyzer, and the result for a 2% agar solution was 150-400 g-cm2.

CONCLUSION

This simple process using inexpensive equipment can be used to produce sheet agar as a food additive. Processes for other uses need to be investigated.

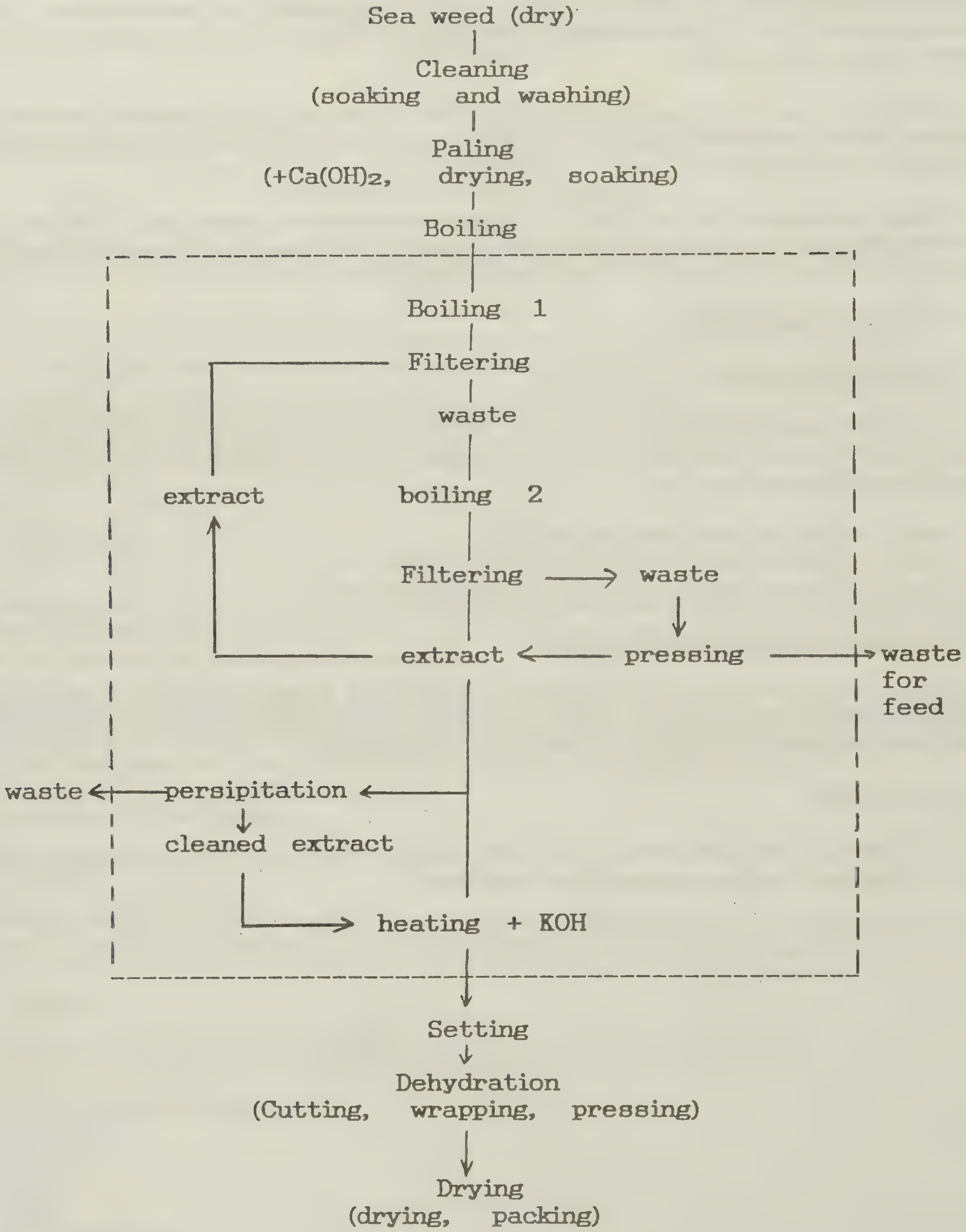
Sea weed (dry)
Cleaning
(soaking and washing)
Paling
(+Ca(OH)2, drying, soaking)
Boiling
Boiling 1
Filtering
waste
extract
boiling 2
Filtering
waste
extract
pressing
waste for feed
waste
persipitation
cleaned extract
heating + KOH
Setting
Dehydration
(Cutting, wrapping, pressing)
Drying
(drying, packing)

Figure 1. Scheme of sheet agar processing

SOME EFFECTS OF PREDRYING TREATMENTS ON *Eucheuma cottonii*

by

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ABSTRACT

The aim of this research was to introduce a cheap and simple method of drying seaweed for carageenan production. The seaweed used was *Eucheuma cottonii*, aged 45 days, harvested from seaweed culture in "Palau Seribu" (Jakarta). Seaweed samples were 1,000 g for each experimental unit. They were dried on "para-para" (similar to a window screen made of bamboo or wire). Samples were dried for 3 days, from 6.00 am to 4.00 pm each day. The experiment was a fully randomized design with 5 treatments and 3 replications.

Five methods of drying were tried:

A. Dipped in fresh water for 6 hours before drying.
B. Packed in transparent PE bags for 6 hours before drying.
C. Packed in black opaque PE bags for 6 hours before drying.
D. Dried over aluminium sheet on para-para.
E. Dried directly on "para-para" as controls.

Parameters used were moisture content of dry seaweed, yield of carageenan and sulphate content of carageenan powder. The best method of drying of seaweed for carageenan was treatment D (samples which were dried over aluminium sheet on "para-para". In the future it might be better if the combination treatments are used.

INTRODUCTION

In 1983, Indonesia produced more than 96,000 tons of seaweed of which 340,000 tons was exported (DGF, 1985). However, the methods of drying are still undeveloped. The aim of this research was to study the effect of some treatments of seaweed before drying, and to find if there was any better method of drying.

Carageenan is produced from dry seaweed, and the yield depends on moisture content. Pretreatments before drying affects the moisture content of dry seaweed, and carageenan yield (Chapman and Chapman, 1980). Carageenan is extracted from dry seaweed using hot fresh water, and sodium hydroxide, and also hydrochloric acid as a pH neutralizing agent (Towle, 1973). As an extractant, to produce carageenan with a high sulphate content, NaOH is better than $Ca(OH)_2$ (Dawes *et al*, 1977). Isopropanol is used to wash carageenan sediments (Moirano, 1977).

Seaweed *Eucheuma cottonii* belongs to order Gigantinales, sub class Floridophycecae, class Rhodophyceae, group Rodophyta (red algae). Rhodophyta, red algae, are coloured algae with an accessory pigment, phycoerythrin, similar to other pigments, such as chlorophyll, phycocyanin, beta-carotene, luteine and zeaxanthine. Rhodophyta produce a starchy reserve polysaccharide, *floridae starch*, which is deposited in the chloroplasts. Other polysaccharides are formed as slimy substances on the outside of the protoplast. The latter are mostly galactans with sulphate ester groups. The most important of these slimy polysaccharides are agar and carageenan. Agar is used in microbiological laboratories for the preparation of solidified nutrient media, and carageenan is used as a thickener in the food industry.

Carageenan is a polysacharide, which is produced as the Na, K or Ca salt. There are various types of carageenan, two of them, kappa and iota carageenan, are important industrially; *Eucheuma cottonii* contains kappa carageenan (Doty, 1973).

METHODS

Eucheuma cottonii, which was 45 days old (maximum content of carageenan) was harvested from the seaweed culture area in Palau Seribu (Jakarta) in the evening. It was cleaned from any other species and dirt. Then it was put on "para-para" (similar to window screen made of bamboo or wire) to remove all attached sea water for 5 hours. Seaweed was then weighed into 5 lots of 1,000 g; each group had different treatments before drying. They were:

A. Dipped in fresh water for 6 hours before drying.
B. Packed in transparent PE bags for 6 hours before drying.
C. Packed in black opaque PE bags for 6 hours before drying.
D. Dried over aluminium sheet on "para-para".
E. Dried directly on "para-para" as control.

All samples were dried under the sun from 6.00 am to 4.00 pm for three days. Then the moisture content of wet and dry seaweed were determined in an oven at 105°C for 3 hours.

$$\text{Moisture content} = \frac{\underline{10 - \text{dry sample weight}}}{10} \times 100\%$$

Carageenan was determined by a simple method, forty grams of samples were extracted with 1,200 ml of hot fresh water at 85°C for 3 hours. Sodium hydroxide (2%) was added to pH 8.0 - 8.5, and hydrochloric acid (1%) was then used to make pH neutral (7). Isopropanol 85% (about 2,000 ml) was used to precipitate and wash the carageenan sediment. After that, the sediment was dried in an over at 60°C for 3 hours. Then it was blended to a powder.

$$\text{Carageenan yield} = \frac{\underline{(\text{dry seaweed weight} : 40) \times \text{carageenan weight})}}{\text{wet seaweed weight}} \times 100$$

The sulphate content of carageenan was determined by digestion method using a Kjeldahl apparatus. Some grams of dry carageenan powder were dried again in an oven at 105°C for 12 hours. Then 0.5 grams was weight and digested with 12 ml hot nitric acid for 30 minutes. Barium chloride (6 ml) was added to the solution and filtered through a Whatman No. 42 filter paper. Barium sulphate on the filter paper was then burned to produce ash.

$$\text{Sulphate content} = \frac{\underline{41.16 \times BaSo_4 \text{ weight}}}{0.5} \times 100\%$$

The experiment was a fully randomized design with 5 treatments and 3 replications. Parameters were moisture content of seaweed, carageenan yield from wet weight, and sulphate content of carageenan.

RESULTS

Moisture content of dry seaweed

There were variations of moisture content depending on treatment (Table 1 and Figure 1).

Table 1. Moisture content of dry seaweed

Treatments	Moisture Content (%)
A	30.35
B	29.52
C	30.65
D	24.42
E	33.68

Moisture content of wet carageenan was 80%

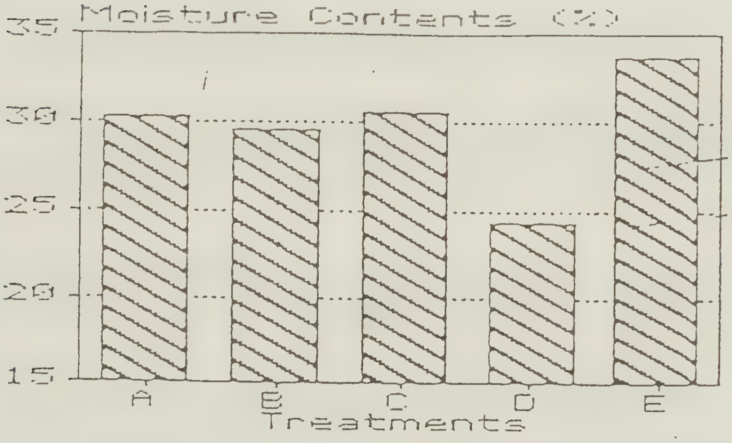

Figure 1. Moisture content diagrams

They were significantly different; the driest was treatment D (24.42%) which was less than the Indonesian seaweed standard for export (25%). Three days were enough to dry *Eucheuma cottonii* if an aluminium sheet was used.

Carageenan Yield

Carageenan yield depends on moisture of seaweed, the extraction methods, and extraction agents (Chapman and Chapman, 1980). It depends on seaweed location of growth, season, and weather and the age of the crop. The yield of carageenan in this experiment are seen in Table 2 and Figure 2.

Table 2. Carageenan Yield of *E. cottonii*

Treatments	Yield of wet weight (%)
A	7.48
B	8.89
C	8.38
D	7.21
E	7.28

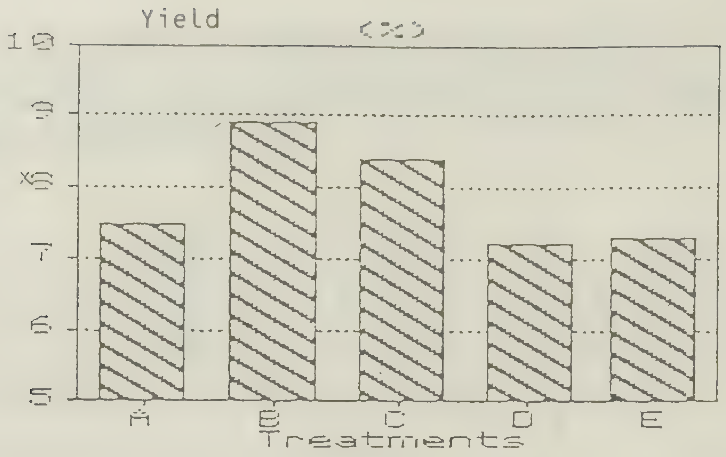

Figure 2. Carageenan yield diagrams

They were insignificantly different. So, all of the treatments gave the same result. However, they were less than the figure of 9.0% of wet weight suggested by Doty (1987).

Sulphate Content

Sulphate content depends on extraction methods and extraction agents (Dawest, 1977). It depends on pre-treatment and drying (Table 3 and Figure 3).

Table 3. Sulphate content of carageenan powder

Treatments	Sulphate content (%)
A	14.64
B	9.22
C	9.67
D	16.48
E	11.19

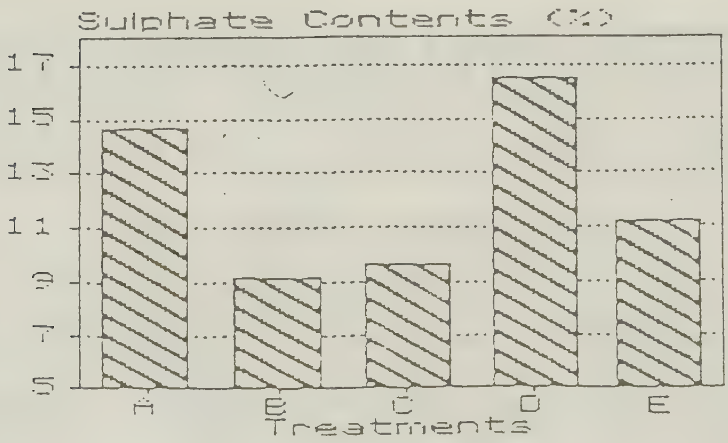

Figure 3. Sulphate content diagrams

Moisture of carageenan powder was 5.0%. They were significantly different. The best was treatment D (16.48%). But this result was still below the minimum FCC standard 18-40% (FCC, 1980).

CONCLUSIONS

1. Drying seaweed on an aluminium sheet on para-para gave a good result for moisture content of dry seaweed, and sulphate content of carageenan.
2. It might be better if a combination of treatments are used in the future.

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DEPURATION OF MUSSELS: A PRELIMINARY TRIAL

by

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ABSTRACT

A depuration unit was designed to reduce microbial contaminant in mussel *(Anadara granosa)*. The unit consisted of a depurating container, plastic boxes to collect the water circulated by pump and a UV sterilizer. Analysis was conducted for TVC and *Vibrio parahaemolyticus.*

Results showed that depuration significantly reduced total viable counts and inhibited vibrio growth. Counts decreased from log 6.55 to 2.72 after 48 hours.. Without depuration the counts reached 8.74.

INTRODUCTION

Although there has not been a major disease outbreak attributed to mussels consumption in Indonesia there is a risk as mussel are filter feeders and are often grown in polluted areas. The can accumulate pathogenic bacteria and other contaminants such as heavy metals, There is a tendency to eat half cooked mussels rendering the consumer liable to *V.cholera*, Hepatitis B and *Salmonella*. Depuration in sterilized water has the potential to reduce contamination. Paranginangin (1985) lists the various methods as chlorination, ozone treatment, membrane filtration and UV light.

MATERIALS AND METHODS

The mussels were collected from a landing place in north Jakarta. They originated from Indramayu, West Java.

The depuration unit was made up of HDPE boxes of 56 x 37 x 18 cm containing 35 litres of water. Each container had two trays with holes to allow the dirt to drop to the bottom. The dirty water was discharged through a hole with the clean water inlet controlled at 3 l/min by a stainless steel tap. The dirty water was collected in two 50 litre HDPE boxes from where it was pumped to the UV sterilization unit with 4 UV lamps.

For depuration the unit was filled with clean sea water and the live mussels put on the trays at a rate of 8 kg/box. The pump and the UN lamps were put on to start the process for a period of 48 hours with the water temperature maintained in the range of 26-28°C. Samples were withdrawn for analysis every 12 hours and compared with undepurated controls.

RESULTS AND DISCUSSION

The results of depuration are shown in Table 1. Starting from a TPC of log 6.55 this decreased by about 1 log cycle every twelve hours to reach log 2.72 after 48 hours. The undepurated control in contrast increased by one log cycle each twelve hours to reach log 8.74 after 48 hours. This result points out the need for depuration, however, a figure of about 5 log cycles after twelve hours implies a longer period is required to meet the minimum standard for human consumption in Indonesia (Ministry of Agriculture, 1988).

Vibrio parahaemolyticus has been increasingly recognized as a course of food-borne disease where seafood is the vector (Nickelson *et al*, 1971). The organism can survive in salt water and mud and grow in laboratory media supplemented with 2.5-3% salt (Benchat 1973, Robach and Hickey, 1978).

The experimental results showed that *Vibrio parahaemolyticus* was not eliminated by depuration but it did not grow. The presumptive MPN after 48 hours was still the same as at the start (0.3 presumptive colonies per gram). In contrast the control rose from a figure of 0.4 MPN/g to 0.9 MPN/g after 48 hours. This suggests that undepurated mussels are unsafe for human consumption.

CONCLUSIONS

Depuration using UV light for water sterilization significantly reduces total viable counts after 48 hours. If depuration is used a period of 12 hours is not sufficient to meet the Indonesian standard. It would be wiser to continue for 48 hours.

Growth of *Vibrio parahaemolyticus* was also inhibited but the organism was not eliminated. Both the TVC and *Vibrio* counts increased substantially in mussels kept for 48 hours without depuration.

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Table 1 Microbial contaminants in mussels during 48 hours of depuration

Time	Samples with no depuration		Samples with depuration	
	TPC/g	*V. Parahaemolyticus* (MPN)/g	TPC/g	*V. Parahaemolyticus* (MPN)/g
0 hr	5.47	conf. 0,4	6.55	presump. 0,3
12 hours	5.65	-	5.45	-
24 hours	6.67	-	3.86	-
36 hours	7.06	-	3.52	-
48 hours	8.74	conf. 0,9	2.72	presump. 0,3

EFFECT OF POTASSIUM HYDROXIDE CONCENTRATION ON SEMI-REFINED CARRAGEENAN

by

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ABSTRACT

Carrageenan was extracted from a red seaweed *(Eucheuma cottonii)* using a semi-refined method with potassium hydroxide solution as a protecting agent to prevent carrageenan leaching out during the extraction. The results showed that 1.5N potassium hydroxide has a good performance as shown by measurement on the viscosity (2778.75 cps); acid insoluble ash (0.038%); gelling point (37.8°C); melting point (57.1°C); sulfate ion (19.04 ppm); 3,6 anhydrogalactose (30.28%) and ash content (17.61%). Compared to other treatments, the 1,5N potassium hydroxide shows an excellent performance.

INTRODUCTION

Carrageenaan is a polysaccharide from red seaweed extensively used as a food additive, especially in dairy products and as a suspending agent in the cosmetic and pharmaceutical industries. Carrageenans can be extracted from some seaweeds such as *Eucheuma sp*; *Chondrus charifus* and *Ahnfeltia concinna*. The carrageenan content of red seaweeds depends on culture areas and environmental quality. In Indonesia, species of red seaweed that have been well known as sources of carrageenan are *Eucheuma spinosum* and *Eucheuma cottonii* which grow in almost all islands. Harvesting of cultured red seaweeds is usually done after 42 days.

Doty (1943) reported that *Eucheuma sp* which was harvested within 1.5 months has a maximum carrageenan content.

There are three main groups of carrageenan: kappa-, lamda- and iota- carrageenan. The three groups differ only in their 3.6-anhydrogalactose contents and the position of the ester sulfate groups in the molecule (Certrudes *et al.*, 1975).

Carrageenan can be extracted by two methods, namely: (a) pure extraction (commonly done with isopropyl alcohol); and (b) semi-refined method by using alkali such as potassium hydroxide and calcium carbonate.

In experiments it was shown that addition of calcium carbonate resulted in considerable carrageenan loss. On the other hand, potassium hydroxide demonstrated a capability to increase viscosity, retain losses of sulfate ions and 3.6 anhydrogalactose during heat treatment.

METHODS AND ANALYSIS

The red seaweed, *Eucheuma cottonii*, was obtained from Pulau Seribu, North Jakarta. It was freshly harvested from a crop cultured for 42 days.

Potassium hydroxide concentrations used for the process were: 0.5; 1.0; 1.5 and 2.0% and heating was carried out at a temperature between 80 - 90°C for two hours. After heating, the mass was macerated in a chopper.

The analytical methods used were: water; ash content and acid insoluble ash AOAC (1984); sulfate content method of Jackson and Candles (1978); Gel strength (g/cm^2), viscosity (cps), melting and gelling point methods of Marine ColloidsInc. (1978).

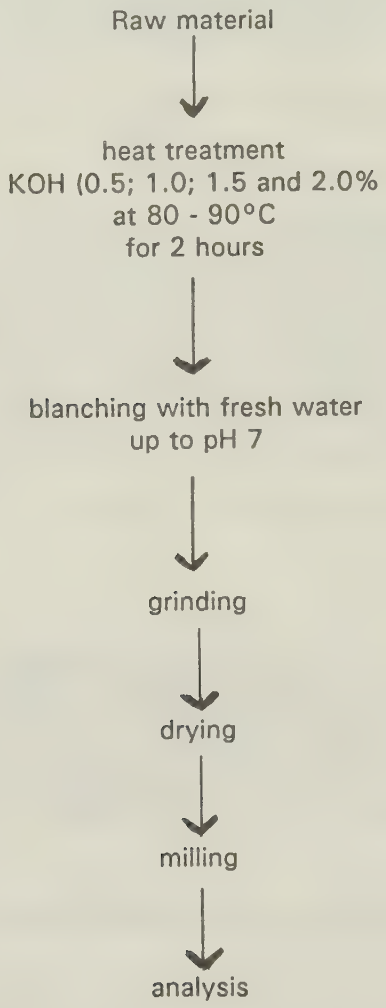

Fig. 1. Diagram of processing a semi-refined carrageenan

RESULTS AND DISCUSSION

a) Viscosity

Generally the higher viscosity in the sample, the higher concentration of carrageenan. Viscosity is closely related with molecular weight and chain length of the molecules. Viscosity, also defined as "Internal friction" or "Resistances to flow", is the key factor in distinguishing between the different types of fluid systems and is a vital factor in the organoleptic acceptability of many food products (Glicksman, 1983). A minimum viscosity of 5 cps is equivalent to a molecular weight of 100,000 (Glicksman, 1983). If the molecular weight drops below 100,000, carrageenan loses its functional properties. All potassium hydroxide treatments showed viscosities higher than 5 cps, i.e., 261.5 cps (KOH 0.5N), 133.7 cps (KOH 1.0N), Fig. 2. All ion-exchanged carrageenan became more viscous in deionized water. The decrease in viscosities of the KOH 0.5N and KOH 1.0N is possibly due to depolymerization of the long chain polymers of carrageenan.

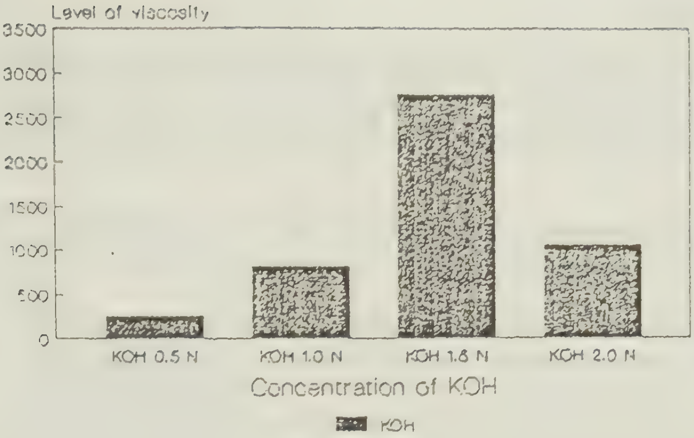

Fig. 2. Viscosity value (cps) for semi-refined carrageenan

b) Ion Sulfate Contents

Carrageenan has a higher sulfate content (15-40%) than other seaweed polysaccharides like agar, alginate and "furcellaran" (FAO, 1979). The sulfate content of the sample is as follows: 19.72 ppm, 17.84 ppm, 18.07 ppm and 19.04 ppm at potassium hydroxide concentrations of 0.5N, 1.0N, 1.5N and 2.0N respectively. Mukti (1987), observed that pH and time of extraction did not affect sulfate contents. He also reported that sulfate content of carrageenan was in a range of 23.97-25.70%. Pemungkas (1987) found sulfate ion content from 19.73-21.07%.

c) Ash content

Ash content of carrageenan consists of minerals like Na^+, K^+, Ca^{++} and iodine, and trace elements like Pb^{++}, Zn^{++}, As^{++}, Fe^{+++}. They are absorbed by the red seaweed (*Eucheuma cottonii*) during growth. Presence of trace elements and minerals depends on the environmental conditions. Treatments with KOH at concentrations of 1.5N and 2.0N have more effect on trace element removal than the other treatments. The maximum reduction was with 1.5N KOH. Fig. 3 shows the ash content after the various treatments. Suryaningrum (1989) reported that average ash content of *Eucheuma cottonii* is about 27.73%. This implies that 1.5N KOH can reduce trace elements and minerals by about 9.9%.

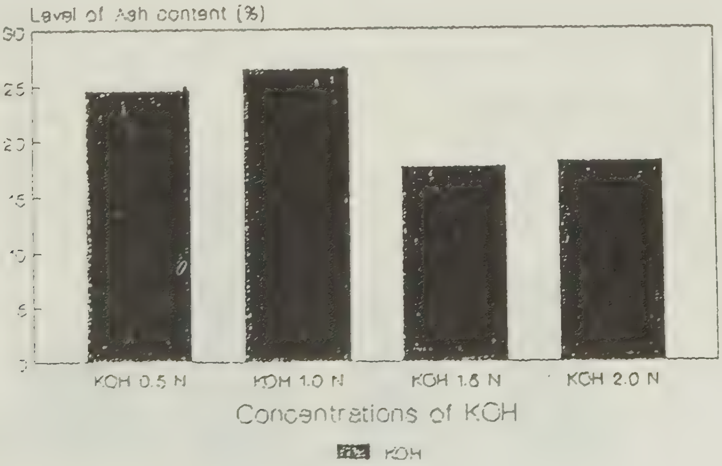

Fig. 3. Ash content (%) for semi-refined carrageenan

d) Gelling and melting point

The gelling and melting points are essential characteristics of the quality of carrageenan as a food ingredient. According to Zabik and Aldrich (1967), gel formation is assisted by positively charged ions such as potassium, sodium or ammonium which aggregate in clusters with negatively charged ester sulfates.

The higher the sulfate content of the carrageenan the more water will be absorbed within the three-dimensional framework of the clusters.

Potassium hydroxide treatment affects both the gelling and melting point in parallel. Increasing the temperature of the gelling point increases the temperature of the melting point, which is always higher than the gelling point. Treatment with 1.5N KOH gave a gelling point of 37.8°C and a melting point of 57.1°C. It is possible that the carrageenan obtains potassium ions from the KOH as below a potassium concentration of 0.007M potassium carrageenans will not gel even at low temperatures (Rocahas and Rinando 1984). Fig. 4 shows the gelling and melting points of the four treatments.

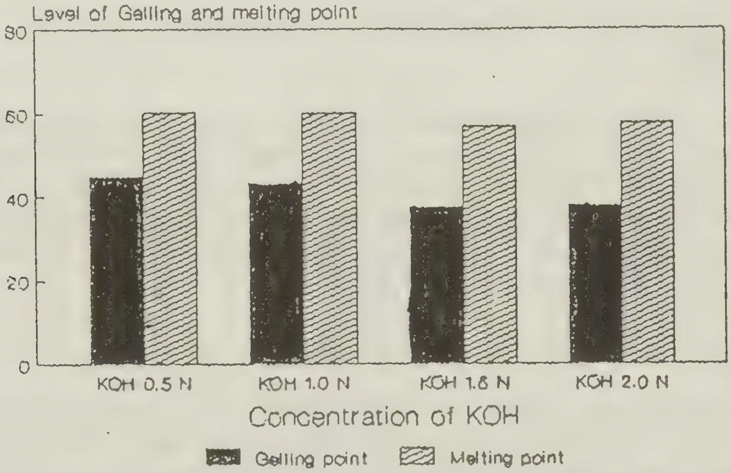

Fig. 4. Gelling and melting point for semi-refined carrageenan

e) Water content

Water content of *Eucheuma cottonii* fluctuates in a range of 17.36-24.39% (Suryaningrum, 1988).

After potassium hydroxide treatment the water content of the carrageenan varied according to Fig. 5. Only the 1.5N KOH treatment had a content over 16% - compared with the FAO, FCC and EEC requirement of 12% maximum.

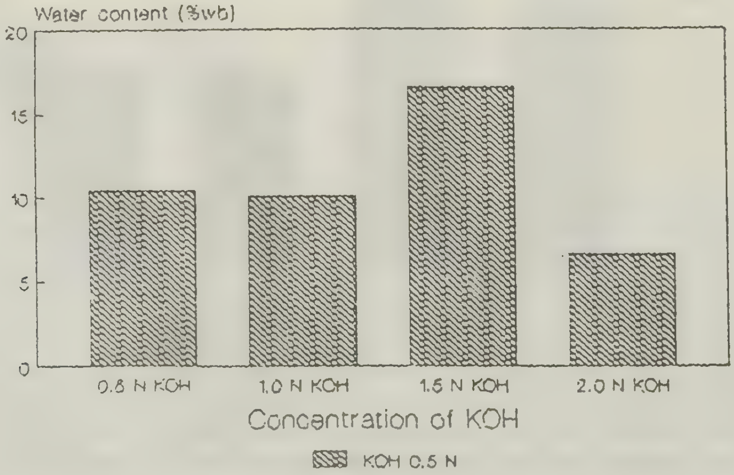

Fig. 5. Water content (% wb) for semi-refined carrageenans

f) Acid insoluble ash

The average acid insoluble ash in all experiments was lower than the standard of 2% maximum.

The treatment apparently removes minerals as shown in Fig. 6 where the 1.5N. KOH treatment has an acid insoluble ash of 0.038%.

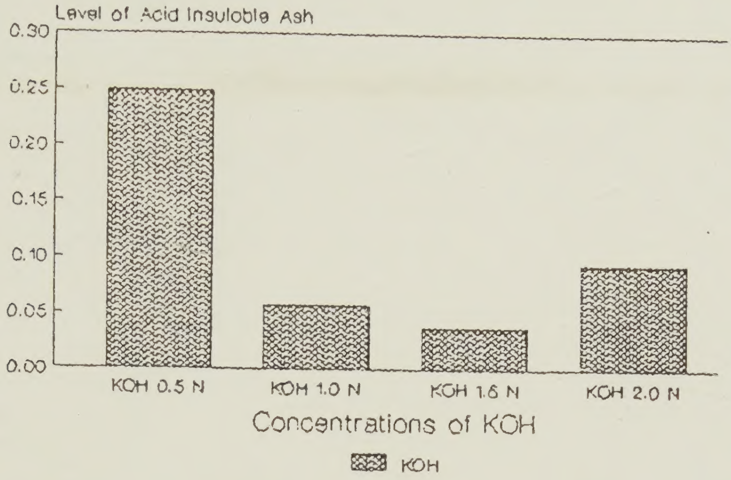

Fig. 6. Acid Insoluble Ash (%) for semi-refined carrageenan

CONCLUSION

Treatment of *Eucheuma cottonii* with hot potassium hydroxide reduces losses of carrageenan and improves quality. Semi-refined carrageenan treated with 1.5N KOH shows good compliance with the FAO, FCC and EEC standards.

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